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(54) **Tissue inhibitor of metalloproteinase type three (TIMP-3)**

Gewebeinhibitor für Metalloproteasen Type 3 (TIMP-3)

Inhibiteur de métalloprotéases d'origine tissulaire du type 3 (TIMP-3)

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Description**Field of the Invention**

5 [0001] The present invention relates in general to metalloproteinase inhibitors and to polynucleotides encoding such factors. In particular, the invention relates to novel mammalian tissue inhibitors of metalloproteinase (herein designated as type three, or "TIMP-3"), to fragments, derivatives, and analogs thereof and to polynucleotides encoding the same. In another aspect, the present invention relates to novel methods of producing such compositions, and methods of using such compositions.

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Background of the Invention

[0002] Connective tissues are maintained in dynamic equilibrium by the opposing effects of extracellular matrix synthesis and degradation. The extracellular connective tissue matrix consists predominantly of collagens, with proteoglycans, fibronectin, laminin and other minor components making up the remainder.

15 [0003] Degradation of the matrix is brought about by the release of neutral metalloproteinases from resident connective tissue cells and invading inflammatory cells that are capable of degrading at physiological pH most of the matrix macromolecules. See Table 1, below. The proteinases include the mammalian tissue collagenases, gelatinases, and proteoglycanases; leukocyte collagenase and gelatinase (Murphy et al., Biochem. J. 283: 289-221 (1982); Hibbs et al.,
20 J. Biol. Chem. 260: 2493-2500 (1985)); macrophage collagenase and elastase (Werb et al., J. Exp. Med. 142: 346-360 (1975); Banda et al., Biochem. J. 193: 589-605 (1981)); and tumour collagenases (Liotta et al., PNAS-USA 76: 2268-2272. (1979); Liotta et al., Biochem. Biophys. Res. Commun. 98: 124-198 (1981); and Salo et al., J. Biol. Chem. 258: 3058-3063 (1983)). For a general review of collagenases and their role in normal and pathological connective
25 tissue turnover see Collagenase in Normal and Pathological Connective Tissues, David E. Woolley and John M. Evanson, eds., John Wiley & Sons Ltd. (1988).

[0004] There are over five different collagen types (I, II, III, IV, V, etc.) which are differentially distributed among tissues. There is considerable homology and structural similarity among the various collagen types. Particular collagenases show some specificity for particular collagen types. See Table 1, below; Matrisian, Trends In Genetics 6: 121-125 (1990). With regard to inhibition of collagenases and other matrix-degrading metalloproteinases, it is possible
30 that, depending on the actual enzymes, substrates, and inhibitory mechanisms, an inhibitor could act on just one, on several, or on all collagenases and metalloproteinases.

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TABLE 1
MATRIX-degrading metalloproteinases

Name(s)	Size (kDa)	Degrades	Ref.
(1) Interstitial collagenase (Type I collagenase) (MMP-1)	52 deduced 52, 57 secreted	I, II, III collagen	Schultz et al., <i>Cancer Res.</i> 48:5539; 5545 (1988)
PMN Collagenase (MMP-8)	75 secreted	I, II, III collagen	Macartney et al., <i>Eur. J. Biochem.</i> 130: 71-78 (1983); Collier et al., <i>J. Biol. Chem.</i> 263:6579-6587 (1988)
(2) 72 kDa Type IV collagenase (72 kDa gelatinase) (MMP-2)	72 secreted	IV, V, VII collagen, fibronectin, gelatins	Wilhelm et al., <i>J. Biol. Chem.</i> 263: 17213-17221 (1989)
92 kDa Type IV collagenase (92 kDa gelatinase) (MMP-9)	78 deduced 92 secreted	IV, V collagen, gelatins	Chin et al., <i>J. Biol. Chem.</i> 260: 12367-12376 (1985)
(3) Stromelysin (transin) (proteoglycanase) (procollagen-activating factor)	53 deduced 57,60 secreted	Proteoglycans, laminin, fibronectin, III, IV, V collagen, gelatins	Nicholson et al., <i>Biochemistry</i> 28: 5195-5203 (1989)
(MMP-3) Stromelysin-2 (transin-2)	53 deduced	III, IV, V collagen, fibronectin, gelatins	Quanin et al., <i>Biochemistry</i> 28: 5327-5333 (1989)
(MMP-10) PGMP-1 (MMP-7) (Small metalloproteinase of uterus)	28 deduced 28 secreted	Gelatins, fibronectin	

The matrix metalloproteinases are divided into three major subclasses, indicated with arabic numerals, on the basis of their substrate specificities.
The enzymes in each class are bold, and alternative names are shown in parentheses. MMP, matrix metalloproteinase; PMN, polymorphonuclear leukocyte.

[0005] The underlying basis of degradative diseases of connective tissue points to the matrix-specific metalloproteinases as having a fundamental role in the etiology of these diseases. Such diseases include dystrophic epidermolysis bullosa; rheumatoid arthritis; corneal, epidermal or gastric ulceration; periodontal disease; emphysema; bone disease; and tumor metastasis or invasion.

[0006] Most studies on connective tissue degradation and diseases involving such degradation have limited the measurement of metalloproteinases to collagenase (the most widely studied of this group of metalloproteinases). It is understood however, that the simultaneous effects of collagenase and the other matrix-degrading metalloproteinases will exacerbate the degradation of the connective tissue over that achieved by collagenase alone.

5 [0007] Specific natural inhibitors of collagenase were discovered in crude medium from cultured connective tissues. A metalloproteinase inhibitor known as TIMP (tissue inhibitor of metalloproteinases) has been studied with regard to physicochemical properties and the biochemistry of its interaction with collagenase, Murphy et al., J. Biochem. 195: 167-170 (1981); Cawston et al., J. Biochem. 211: 313-318 (1983); Stricklin et al., J. Biol. Chem. 258: 12252-12258 (1983), and DNA encoding it has been isolated, Docherty et al., Nature 318: 65-69 (1985); Carmichael et al., PNAS-USA 83: 2407-2411 (1986). In an in vitro cell culture model of tumor cell migration through a natural basement membrane, TIMP was able to arrest migration of a collagenase-secreting tumor cell line, Thorgeirsson et al., J. Natl. Canc. Inst. 69: 1049-1054 (1982). In vivo mouse lung colonization by murine B16-F10 melanoma cells was inhibited by injections of TIMP, Schultz et al., Cancer Research 48: 5539-5545 (1988). European Patent Publication No. EP O 189 784 also relates to TIMP.

10 [0008] McCartney et al., Eur. J. Biochem. 130: 79-83 (1983) reported the purification of a metalloproteinase inhibitor from human leukocytes.

[0009] DeClerck et al., Cancer Research 46: 3580-3586 (1986) described the presence of two inhibitors of collagenase in conditioned medium from bovine aortic endothelial cells.

15 [0010] Murray et al., J. Biol. Chem. 261: 4154-4159 (1986) reported the purification and partial amino acid sequence of a bovine cartilage-derived collagenase inhibitor.

[0011] Langley, et al., EP O 398 753 ("Metalloproteinase Inhibitor," published November 22, 1990) discloses a novel metalloproteinase inhibitor and analogs, polynucleotides encoding the same, methods of production, pharmaceutical compositions, and methods of treatment. The polypeptide of Figure 2 therein has been referred to as TIMP-2, designating a molecule distinct from TIMP-1, *supra*. EP O 398 753 describes both bovine and human recombinant TIMP-2.

20 [0012] Staskus et al., J. Biol. Chem. 266: 449-454 (1991) reports a 21 kDa avian metalloproteinase inhibitor obtained from chicken fibroblasts. The authors note the biochemical similarities with other members of the TIMP and TIMP-2 group of proteins and state that the avian material may be a TIMP variant or may represent a third protein within the metalloproteinase inhibitor family. (This material is referred to herein as "ChIMP-3")

25 [0013] Pavloff et al., J. Biol. Chem. 267: 17321-17326 (1992) discloses the cDNA and primary structure of a metalloproteinase inhibitor from chicken embryo fibroblasts.

[0014] Yang et al., PNAS-USA 89: 10676-10680 (1992) reports on the role of a 21 kDa protein chicken TIMP-3.

30 [0015] Herron et al., J. Biol. Chem. 261: 2814-2818 (1986) disclose three metalloproteinase inhibitors produced by rabbit brain capillary endothelial cells.

[0016] Kishnani et al., FASEB Journal 7, page A371, Abstract 2148 (1993) describe the identification of three metalloproteinase inhibitors in the extracellular matrix of cultured human cells.

35 [0017] The present work relates to a third type of metalloproteinase inhibitor polypeptides. In one aspect, the present invention involves the cloning of recombinant human TIMP-3 nucleic acid and expression thereof.

Summary of the Invention

40 [0018] According to the present invention, a class of novel tissue inhibitors of metalloproteinase are provided. For convenience, the present polypeptides are referred to as "TIMP-3," as these polypeptides represent a new class of members of the tissue inhibitors of metalloproteinases. Also provided are DNA sequences coding for all or part of the present TIMP-3's, vectors containing such DNA sequences, and host cells transformed or transfected with such vectors.

45 [0018] Also comprehended by the invention are methods of producing recombinant TIMP-3's, and methods of treating disorders. Additionally, pharmaceutical compositions including TIMP-3's and antibodies selectively binding TIMP-3's are provided.

Brief Description of the Drawings

50 [0019] Figure 1 shows the cDNA sequence and amino acid sequence of a recombinant human tissue inhibitor of metalloproteinase type 3 ("TIMP-3"). The entire 1240 base pair sequence encoding a full-length polypeptide of 211 amino acids is presented. A hydrophobic leader sequence is found at position -23 to -1. The initial cysteine of the mature protein is numbered +1. The amino acids corresponding to the degenerate oligonucleotides which identified the original PCR products are underlined, except that the oligo corresponding to YTIK was used analytically to confirm the identity of the PCR products prior to sequencing. A potential glycosylation site is italicized. A variant polyadenylation signal sequence is marked with asterisks. (The abbreviations used herein for amino acids, either single letter or triple letter abbreviations, and nucleic acids are those conventionally used, as in Stryer, Biochemistry, 3d ed. 1988, W.H.

Freeman, N.Y., inside back cover.)

[0020] Figure 2 is a photograph of an agarose gel of first-strand cDNA PCR products, which demonstrate amplification of human nucleic acid. Lane 1 presents PCR products from human fetal kidney first strand cDNA primed with primers 449-5 (Seq. ID No. 1) and 480-27 (Seq. ID No. 2). Lane 2 presents the results of PCR amplification of fetal kidney first strand cDNA primed with primers 449-15 (Seq. ID No. 1) and 480-28 (Seq. ID No. 3). Lane 3 is the PCR kit (Perkin-Elmer-Cetus) control. Lane 4 is TIMP-2 DNA primed with primers 449-15 (Seq. ID No. 1) and 480-27 (Seq. ID No. 2). Lane 5 is molecular weight markers.

[0021] Figure 3 is a photograph of a silver stained SDS-PAGE gel containing material as follows: Lane 1, molecular weight markers; lane 2, TIMP-2, reduced; lane 3, blank; lane 4, *E. coli* derived TIMP-3 of Figure 1, reduced, pre-dialysis; lane 5, *E. coli* derived TIMP-3 of Figure 1, reduced, post-dialysis, lanes 6, 7, 8, blank; lane 9, *E. coli* derived TIMP-3 of Figure 1, unreduced, pre-dialysis; lane 10, *E. coli* derived TIMP-3 of Figure 1, unreduced, post-dialysis.

[0022] Figure 4 is a comparison table of human TIMP-3 amino acid sequence of Figure 1 with other TIMP family members. The numbering begins with the first cysteine of the mature protein. As can be seen, the alignment contains gaps for some TIMP family members. The numbering used here is consistent for the numbering used for the recombinant human TIMP-3 of Figure 1. Boldface letters indicate conserved amino acids; asterisks represent potential glycosylation sites of TIMP-1; underlined letters indicate potential glycosylation sites of TIMP-3; the left bracket indicates the beginning of the mature proteins. A bullet (·) indicates those amino acids which are unique to recombinant human TIMP-3. The amino acid sequences were found in the literature as follows: Bovine TIMP-1, Freudenstein et al., Biochem. Physic. Res. Comm. 171: 250-256 (1990); Human TIMP-1, Docherty et al., Nature 318: 65-69 (1985); Rabbit TIMP-1, Horowitz et al., J. Biol. Chem. 264: 7092-7095 (1989); Mouse TIMP-1, Edwards et al., Nucleic Acid. Res. 14: 8863-8878 (1986); Johnson et al., Mol. Cell. Biol. 7: 2821-2829 (1978); Gewert et al., EMBO 6: 651-657 (1987); Bovine TIMP-2, Boone et al., PNAS-USA 87: 2800-2804 (1990); Human TIMP-2, Boone et al., PNAS-USA 87: 2800-2804 (1990); Mouse TIMP-2, Shimizu et al., Gene 114: 291-292 (1992); Chicken TIMP-3, Pavloff et al., J. Biol. Chem. 267: 17321-17326 (1992). Unless otherwise indicated, these sequences referred to from time to time herein were found in these references.

[0023] Figure 5 is a comparison table of the amino acid sequence for the chicken metalloproteinase inhibitor of Staskus et al., J. Biol. Chem. 266: 449-454 (1991) and the recombinant human TIMP-3 of Figure 1. A solid line between amino acids indicates identity, double dots indicates similarity. A single dot indicates a lesser degree of similarity, and no dot indicates total difference, as described by Grivkov et al., Nucl. Aud. Res. 14: 6745-6763 (1986).

[0024] Figure 6 shows the overall homology between the Figure 1 nucleic acid sequence encoding TIMP-3 and that encoding ChiMP-3.

[0025] Figure 7 shows the maximal homology between the Figure 1 nucleic acid sequence encoding TIMP-3 and that encoding ChiMP-3.

[0026] Figure 8 shows the amino acid sequence alignment of human recombinant TIMP-3 of Figure 1 and human TIMP-2.

[0027] Figure 9 shows the overall homology of the Figure 1 nucleic acid sequence of human recombinant TIMP-3 and that encoding human TIMP-2.

[0028] Figure 10 shows the maximal homology regions of the Figure 1 nucleic acid sequence encoding human recombinant TIMP-3 and that encoding human TIMP-2.

[0029] Figure 11 shows the amino acid sequence alignment of human recombinant TIMP-3 of Figure 1 and human TIMP-1.

[0030] Figure 12 shows the overall homology of the Figure 1 nucleic acid sequence encoding human recombinant TIMP-3 and that encoding human TIMP-1.

[0031] Figure 13 shows the maximal homology regions of the Figure 1 nucleic acid sequence encoding human recombinant TIMP-3 and that encoding human TIMP-1.

[0032] Figures 14 A and B shows Northern blot analyses performed on RNAs from a variety of cells, using a TIMP-3 DNA fragment as a probe.

[0033] Figure 15 shows a modified zymogram. Lane 1 (from the left hand side) contains a protein molecular weight standard (see Figure 3). Lane 2 is a control lane containing conditioned medium with collagenases (72 kDa and interstitial collagenases, pAPMA activated). ("Coll" refers to interstitial collagenase.) Lane 3 contains TIMP-2. Lane 4 contains a TIMP-2 analog lacking the six C-terminal cysteines. Lanes 5, 6, and 7 contain *E. coli* derived TIMP-3 of Figure 1, lane 5 being undiluted and lanes 6 and 7 being consecutive 2-fold serial dilutions. As can be seen, the lack of a clear zone at the location where the control (lane 2) showed clearing indicates that TIMP-3 inhibits collagenase activity.

[0034] Figure 16 shows the cDNA and amino acid sequence of variants obtained using the present method.

[0035] Figure 17 shows an illustration of a proposed secondary structure of members of the TIMP family of proteins.

[0036] Numerous aspects and advantages of the invention will be apparent to those skilled in the art upon consideration of the following detailed description which provides illustrations of the practice of the invention in its presently preferred embodiments.

Detailed Description of the Invention

[0037] According to the present invention, novel metalloproteinase inhibitors (herein called, collectively, TIMP-3) and DNA sequences coding for all or part of such TIMP-3 are provided. Such sequences include the incorporation of codons "preferred" for expression by selected nonmammalian hosts; the provision of sites for cleavage by restriction endonuclease enzymes; and the provision of additional initial, terminal or intermediate DNA sequences which facilitate construction of readily expressed vectors. The present invention also provides DNA sequences coding for polypeptide analogs or derivatives of TIMP-3 which differ from naturally-occurring forms in terms of the identity or location of one or more amino acid residues (i.e., deletion analogs containing less than all of the residues specified for TIMP-3; substitution analogs, wherein one or more residues specified are replaced by other residues; and addition analogs wherein one or more amino acid residues is added to a terminal or medial portion of the polypeptide) and which share some or all the biological properties of mammalian TIMP-3.

[0038] Novel nucleic acid sequences of the invention include sequences useful in securing expression in prokaryotic or eukaryotic host cells of polypeptide products having at least a part of the primary structural conformation and one or more of the biological properties of recombinant human TIMP-3. The nucleic acids may be purified and isolated, so that the desired coding region is useful to produce the present polypeptides, for example, or for diagnostic purposes, as described more fully below. DNA sequences of the invention specifically comprise: (a) the DNA sequence set forth in Figure 1 (and complementary strands); (b) a DNA sequence which hybridizes (under hybridization conditions disclosed in the cDNA library screening section below, or equivalent conditions or more stringent conditions) to the DNA sequence in Figure 1 or to fragments thereof; and (c) a DNA sequence which, but for the degeneracy of the genetic code, would hybridize to the DNA sequence in Figure 1. Also contemplated are fragments of (a), (b) or (c) above which are at least long enough to selectively hybridize to human genomic DNA encoding TIMP-3, under conditions disclosed for the cDNA library screening, below. Specifically comprehended in parts (b) and (c) are genomic DNA sequences encoding allelic variant forms of human TIMP-3 and/or encoding TIMP-3 from other mammalian species, and manufactured DNA sequences encoding TIMP-3, fragments of TIMP-3, and analogs of TIMP-3 which DNA sequences may incorporate codons facilitating transcription and translation of messenger RNA in microbial hosts. Such manufactured sequences may readily be constructed according to the methods of Alton et al., PCT published application WO 83/04053.

[0039] Genomic DNA encoding the present TIMP-3's may contain additional non-coding bases, or introns, and such genomic DNAs are obtainable by hybridizing all or part of the cDNA, illustrated in Figures 1 and 16, to a genomic DNA source, such as a human genomic DNA library. Such genomic DNA will encode functional TIMP-3 polypeptide; however, use of the cDNAs may be more practicable in that, since only the coding region is involved, recombinant manipulation is facilitated.

[0040] According to another aspect of the present invention, the DNA sequences described herein which encode TIMP-3 polypeptides are valuable for the information which they provide concerning the amino acid sequence of the mammalian protein which have heretofore been unavailable. Put another way, DNA sequences provided by the invention are useful in generating new and useful viral and circular plasmid DNA vectors, new and useful transformed and transfected prokaryotic and eukaryotic host cells (including bacterial and yeast cells and mammalian cells grown in culture), and new and useful methods for cultured growth of such host cells capable of expression of TIMP-3 and its related products.

[0041] The DNA provided herein (or corresponding RNAs) may also be used for gene therapy for, example, treatment of emphysema. For example, transgenic mice overexpressing collagenase exhibit symptoms pulmonary emphysema, D'Armiento et al., *Cell* 71: 955-961 (1992), indicating that inhibition of collagenase may ameliorate some of the symptoms of emphysema. Currently, vectors suitable for gene therapy (such as retroviral or adenoviral vectors modified for gene therapy purposes and of purity and pharmaceutical acceptability) may be administered for delivery into the lung. Such vectors may incorporate nucleic acid encoding the present polypeptides for expression in the lung. Additionally, one may use a mixture of such vectors, such as those containing genes for one or more TIMPs, elastase inhibitors or other proteins which ameliorate the symptoms of emphysema. Gene therapy may involve a vector containing more than one gene for a desired protein.

[0042] Alternatively, one may use no vector so as to facilitate relatively stable presence in the host. For example, homologous recombination may facilitate integration into a host genome. The nucleic acid may be placed within a pharmaceutically acceptable carrier to facilitate cellular uptake, such as a lipid solution carrier (e.g., a charged lipid), a liposome, or polypeptide carrier (e.g., polylysine). A review article on gene therapy is Verma, *Scientific American*, November 1990, pages 68-84.

[0043] As mentioned above, target cells may be within the lungs of the recipient, but other target cells may be bone marrow cells, blood cells, liver (or other organ) cells, muscle cells, fibroblasts, or other cells. The desired nucleic acid may be first placed within a cell, and the cell may be administered to a patient (such as a transplanted tissue) or the desired nucleic acid may be administered directly to the patient for uptake *in vivo*.

[0044] The cells to be transferred to the recipient may be cultured using one or more factors affecting the growth or proliferation of such cells, as for example, SCF.

[0045] Administration of DNA of the present invention to the lung may be accomplished by formation of a dispersion of particles, or an aerosol. Typically some type of bulking agent will be involved, and a carrier, such as a lipid or polypeptide. These materials must be pharmaceutically acceptable. One may use a nebulizer for such delivery, such as an ultrasonic or dry powder nebulizer. Alternatively, one may use a propellant based system, such as a metered dose inhaler, which may deliver liquid or a suspension of particles.

[0046] For gene therapy dosages, one will generally use between one copy and several thousand copies of the present nucleic acid per cell, depending on the vector, the expression system, the age, weight and condition of the recipient and other factors which will be apparent to those skilled in the art.

[0047] DNA sequences of the invention are also suitable materials for use as labeled probes in isolating human genomic DNA encoding TIMP-3, as mentioned above, and related proteins as well as cDNA and genomic DNA sequences of other mammalian species. DNA sequences may also be useful in various alternative methods of protein synthesis (e.g., in insect cells) or, as described above, in genetic therapy in humans and other mammals. DNA sequences of the invention are expected to be useful in developing transgenic mammalian species which may serve as eucaryotic "hosts" for production of TIMP-3 and TIMP-3 products in quantity. See, generally, Palmiter et al., *Science* 222: 809-814 (1983).

[0048] Also, one may prepare antisense nucleic acids against the present DNAs. Compare, Khokho et al., *Science* 243: 947-950 (1989), whereby antisense RNA inhibitor of TIMP conferred oncogenicity on Swiss 3T3 cells. Antisense nucleic acids may be used to modulate or prevent expression of endogenous TIMP-3 nucleic acids.

[0049] The present invention provides purified and isolated polypeptide products having part or all of the primary structural conformation (i.e., continuous sequence of amino acid residues) and one or more of the biological properties (e.g., immunological properties and *in vitro* biological activity) and physical properties (e.g., molecular weight) of naturally-occurring mammalian TIMP-3 including allelic variants thereof. The term "purified and isolated" herein means substantially free of unwanted substances so that the present polypeptides are useful for an intended purpose. For example, one may have a recombinant human TIMP-3 substantially free of other human proteins or pathological agents. These polypeptides are also characterized by being the a product of mammalian cells, or the product of chemical synthetic procedures or of procaryotic or eucaryotic host expression (e.g., by bacterial, yeast, higher plant, insect and mammalian cells in culture) of exogenous DNA sequences obtained by genomic or cDNA cloning or by gene synthesis. The products of expression in typical yeast (e.g., *Saccharomyces cerevisiae*) or procaryote (e.g., *E. coli*) host cells are free of association with any mammalian proteins. The products of expression in vertebrate (e.g., non-human mammalian (e.g. COS or CHO) and avian) cells are free of association with any human proteins. Depending upon the host employed, and other factors, polypeptides of the invention may be glycosylated with mammalian or other eucaryotic carbohydrates or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue (at position -1 with respect to the first amino acid residue of the polypeptide).

[0050] In addition to naturally-occurring allelic forms of TIMP-3, the present invention also embraces other TIMP-3 products such as polypeptide analogs of TIMP-3 and fragments of TIMP-3. Following the procedures of the above noted published application by Alton et al. (WO 83/04053), one can readily design and manufacture genes coding for microbial expression of polypeptides having primary conformations which differ from that herein specified for in terms of the identity or location of one or more residues (e.g., substitutions, terminal and intermediate additions and deletions). Alternately, modifications of cDNA and genomic genes may be readily accomplished by well-known site-directed mutagenesis techniques and employed to generate analogs and derivatives of TIMP-3. Such products would share at least one of the biological properties of mammalian TIMP-3 but may differ in others. As examples, projected products of the invention include those which are foreshortened by e.g., deletions; or those which are more stable to hydrolysis (and, therefore, may have more pronounced or longer lasting effects than naturally-occurring); or which have been altered to delete one or more potential sites for glycosylation (which may result in higher activities for yeast-produced products); or which have one or more cysteine residues deleted or replaced by, e.g., alanine or serine residues and are potentially more easily isolated in active form from microbial systems; or which have one or more tyrosine residues replaced by phenylalanine and bind more or less readily to target proteins or to receptors on target cells. Also comprehended are polypeptide fragments duplicating only a part of the continuous amino acid sequence or secondary conformations within TIMP-3, which fragments may possess one activity of mammalian TIMP-3 (e.g., immunological activity) and not others (e.g., metalloproteinase inhibiting activity).

[0051] The present TIMP-3 may bind to the extracellular matrix, a characteristic not shared by TIMP-1 and TIMP-2. Thus, polypeptides exhibiting only a part of the continuous amino acid sequence or secondary conformations within TIMP-3 possessing the ability to bind to the extracellular matrix are also specifically contemplated herein.

[0052] It is noteworthy that activity is not necessary for any one or more of the products of the invention to have therapeutic utility (see, Weiland et al., *Blut* 44: 173-175 (1982) or utility in other contexts, such as in assays of TIMP-3 antagonism. Competitive antagonists may be quite useful in, for example, cases of overproduction of TIMP-3.

[0053] Of applicability to TIMP-3 fragments and polypeptide analogs of the invention are reports of the immunological activity of synthetic peptides which substantially duplicate the amino acid sequence extant in naturally-occurring proteins, glycoproteins and nucleoproteins. More specifically, relatively low molecular weight polypeptides have been shown to participate in immune reactions which are similar in duration and extent to the immune reactions of physiologically significant proteins such as viral antigens, polypeptide hormones, and the like. Included among the immune reactions of such polypeptides is the provocation of the formation of specific antibodies in immunologically active animals. See, e.g., Lerner et al., *Cell* 23: 309-310 (1981); Ross et al., *Nature* 294: 654-656 (1981); Walter et al., *PNAS-USA* 77: 5197-5200 (1980); Lerner et al., *PNAS-USA*, 78: 3403-3407 (1981); Walter et al., *PNAS-USA* 78: 4882-4886 (1981); Wong et al., *PNAS-USA* 79: 5322-5326 (1982); Baron et al., *Cell* 28: 395-404 (1982); Dressman et al., *Nature* 295: 185-186 (1982); and Lerner, *Scientific American* 248: 66-74 (1983). See, also, Kaiser et al. *Science* 223: 249-255 (1984) relating to biological and immunological activities of synthetic peptides which approximately share secondary structures of peptide hormones but may not share their primary structural conformation.

[0054] One type of analog is a truncated TIMP-3 having capacity to bind to the zinc binding domain of collagenase. For example, the zinc binding domain on interstitial collagenase is located at amino acids 218, 222 and 228 at the pro enzyme. Goldberg, G.I., *J. Biol. Chem.* 261: 660-6605 (1986). The zinc binding domain of the 72 kDa species of procollagenase is located at amino acids 403-407. Collier et al., *Genomics* 9: 429-434 (1991). The zinc binding domain of the 92 kDa species of procollagenase is at amino acids 401-405. Van Ranst et al., *Cytokine* 3: 231-239 (1991). Interestingly, the zinc binding domain is fairly well conserved among enzymes: H E F G H (92 kDa collagenase), H E F G H (72 kDa collagenase) and H E L G H (interstitial collagenase). Thus, the motif for zinc binding is H E X G H wherein X is either F or L. A selective binding molecule, such as an antibody or small molecule would block such zinc binding and therefore inhibit enzymatic activity. (The term "selective binding molecule" as used here indicating a composition which selectively binds to its target.) One may prepare a monoclonal antibody or a recombinant antibody, for example.

[0055] TIMP-2 deletion analogs have been prepared which have retained the ability to inhibit metalloproteinase activity. Willenbrock et al., *Biochemistry* 32: 4330-4337 (1993). For TIMP-2, the C-terminus was shortened to delete six C-terminal cysteines (three disulfide-bonded loops). Thus, in view of the homology among the various zinc binding domains, one could prepare analogous TIMP-3 analogs with similarly shortened C-terminal sequences. The TIMP-3 analog 1-121 (using the numbering of Figure 1 herein) includes the first six cysteines residues, but not the last six. One may optionally lengthen the C-terminus up to the full length molecule of 188 amino acids. Such analogs may also be prepared for any species, such as ChIMP-3.

[0056] This is further demonstrated below in the examples, as a TIMP-2 deletion variant is shown to inhibit interstitial collagenase. (Example 3 below). The zinc binding domain of interstitial collagenase is similarly situated as that of the 72 kDa species collagenase (which was shown by Willenbrock et al., *supra*, to be affected by the TIMP-2 truncated analogs).

[0057] Also, since it is apparent that the C-terminus is not necessary for enzyme inhibition activity, one may chemically modify the C-terminus. One may desire, for example, a sustained release preparation whereby one or more polymer molecules such as polyethylene glycol molecules are attached. Other chemical modifications include attachment of an additional polypeptide for the creation of a fusion molecule. Thus, another aspect of the present invention is chemically modified TIMP-3.

[0058] The present invention also includes that class of polypeptides coded for by portions of the DNA complementary to the protein-coding strand of the human cDNA or genomic DNA sequences of TIMP-3 i.e., "complementary inverted proteins" as described by Tramontano et al. *Nucleic Acid Res.* 12: 5049-5059 (1984). Polypeptides or analogs thereof may also contain one or more amino acid analogs, such as peptidomimetics.

[0059] Also comprehended by the invention are pharmaceutical compositions comprising effective amounts of polypeptide products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers useful in TIMP-3 therapy. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimerosal, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); covalent attachment of polymers such as polyethylene glycol to the protein (as discussed *supra*, see, for example U.S. patent 4,179,337; incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Such compositions will influence the physical state, stability, rate of *in vivo* release, and rate of *in vivo* clearance of TIMP-3. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712.

[0060] Generally, an effective amount of the present TIMP-3 polypeptides will be determined by the age, weight and condition or severity of disease of the recipient. See, Remingtons Pharmaceutical Sciences, *supra*, at pages 697-773, herein incorporated by reference. Typically, a dosage of between about 0.001g/kg body weight to about 1g/kg body weight, may be used, but more or less, as a skilled practitioner will recognize, may be used. For local (i.e., non-systemic)

applications, such as topical applications, the dosing may be between about 0.001g/cm² to about 1g/cm². Dosing may be one or more times daily, or less frequently, and may be in conjunction with other compositions as described herein. It should be noted that the present invention is not limited to the dosages recited herein.

[0061] A plurality of agents act in concert in order to maintain the dynamic equilibrium of the extracellular matrix and tissues. In treatment of conditions where the equilibrium is skewed, one or more of the other agents may be used in conjunction with the present TIMP-3. These other agents may be co-administered or administered in seriatim, or a combination thereof. Generally, these other agents may be selected from the list consisting of the metalloproteinases, serine proteases, inhibitors of matrix degrading enzymes, intracellular enzymes, cell adhesion modulators, and factors regulating the expression of extracellular matrix degrading proteinases and their inhibitors. While specific examples are listed below, one skilled in the art will recognize other agents performing equivalent functions, including additional agents, or other forms of the listed agents (such as those produced synthetically, via recombinant DNA techniques, and analogs and derivatives).

[0062] Metalloproteinases and serine proteases degrade the extracellular matrix, as discussed above. Thus, use of enzymes in therapy may be to counteract effects of the present TIMP-3, which inhibits such degradation. Enzymes include collagenases, PMN (polymorphonuclear leukocyte) collagenase, stromelysin I, II/transin, matrilysin, invadolin, putative metalloproteinase (PUMP-1), urokinase type plasminogen activator (UPA), tissue plasminogen activator (TPA), and plasmin. PD-ECCG may also be used.

[0063] Other degradation inhibitors may also be used if increased or more specific prevention of extracellular matrix degradation is desired. Inhibitors may be selected from the group consisting of α_2 macroglobulin, pregnancy zone protein, ovostatin, α_1 -proteinase inhibitor, α_2 -antiplasmin, aprotinin, protease nexin-1, plasminogen activator inhibitor (PAI)-1, PAI-2, TIMP-1, and TIMP-2. Others may be used, as one skilled in the art will recognize.

[0064] Intracellular enzymes may also be used in conjunction with the present TIMP-3. Intracellular enzymes also may affect extracellular matrix degradation, and include lysosomal enzymes, glycosidases and cathepsins.

[0065] Cell adhesion modulators may also be used in combination with the present TIMP-3. For example, one may wish to modulate cell adhesion to the extracellular matrix prior to, during, or after inhibition of degradation of the extracellular matrix using the present TIMP-3. Cells which have exhibited cell adhesion to the extracellular matrix include osteoclasts, macrophages, neutrophils, eosinophils, killer T cells and mast cells. Cell adhesion modulators include peptides containing an "RGD" motif or analog or mimetic antagonists or agonists.

[0066] Factors regulating expression of extracellular matrix degrading proteinases and their inhibitors include cytokines, such as IL-1 and TNF- α , TGF- β , glucocorticoids, and retinoids. Other growth factors effecting cell proliferation and/or differentiation may also be used if the desired effect is to inhibit degradation of the extracellular matrix using the present TIMP-3, in conjunction with such cellular effects. For example, during inflammation, one may desire the maintenance of the extracellular matrix (via inhibition of enzymatic activity) yet desire the production of neutrophils; therefore one may administer G-CSF. Other factors include erythropoietin, interleukin family members, SCF, M-CSF, IGF-I, IGF-II, EGF, FGF family members such as KGF, PDGF, and others. One may wish additionally the activity of interferons, such as interferon alpha's, beta's, gamma's, or consensus interferon. Intracellular agents include G-proteins, protein kinase C and inositol phosphatases. While the field of inflammation research is presently under development, and the precise interactions of the described compositions in vivo is not thoroughly understood, the use of the present polypeptides may provide therapeutic benefit with one or more agents involved in inflammation therapy.

[0067] Cell trafficking agents may also be used. For example, inflammation involves the degradation of the extracellular matrix, and the movement, or trafficking of cells to the site of injury. Prevention of degradation of the extracellular matrix may prevent such cell trafficking. Use of the present TIMP-3 in conjunction with agonists or antagonists of cell trafficking-modulation agents may therefore be desired in treating inflammation. Cell trafficking-modulating agents may be selected from the list consisting of endothelial cell surface receptors (such as E-selectins and integrins); leukocyte cell surface receptors (L-selectins); chemokines and chemoattractants. For a review of compositions involved in inflammation, see Carlos et al., Immunol. Rev. 114: 5-28 (1990), which is herein incorporated by reference.

[0068] Moreover, compositions may include neu differentiation factor, "NDF," and methods of treatment may include the administration of NDF before, simultaneously with, or after the administration of TIMP-3. NDF has been found to stimulate the production of TIMP-2, and the combination of NDF, TIMP -1, -2 and/or -3 may provide benefits in treating tumors.

[0069] Polypeptide products of the invention may be "labeled" by association with a detectable marker substance (e.g., radiolabeled with ¹²⁵I) to provide reagents useful in detection and quantification of TIMP-3 in solid tissue and fluid samples such as blood or urine. Nucleic acid products of the invention may also be labeled with detectable markers (such as radiolabels and non-isotopic labels such as biotin) and employed in hybridization processes to locate the human TIMP-3 gene position and/or the position of any related gene family in a chromosomal map. Nucleic acid sequences which selectively bind the human TIMP-3 gene are useful for this purpose. They may also be used for identifying human TIMP-3 gene disorders at the DNA level and used as gene markers for identifying neighboring genes and their disorders. Contemplated herein are kits containing such labelled materials.

[0070] The TIMP-3 compositions described herein modify the pathogenesis and provide a beneficial therapy for diseases of connective tissues characterized by matrix degradation. Also, the present TIMP-3 compositions may be useful in the treatment of any disorder where excessive matrix loss is caused by metalloproteinase activity. The TIMP-3 compositions may be used alone or in conjunction with one or more of the agents discussed herein.

5 [0071] Polypeptide products of the present invention are useful, alone or in combination with other drugs, in the treatment of various disorders such as dystrophic epidermolysis bullosa where the disease is linked to the overproduction of collagenase, Bauer et al., *J. Exp. Med.* 148: 1378-1387 (1978). The products of the present invention may also be useful in the treatment of rheumatoid arthritis. Evanson et al. *J. Clin. Invest.* 47: 2639-2651 (1968) noted that large amounts of collagenase are produced, in culture, by excised rheumatoid synovial tissue, this led to immunological studies by Woolley et al., *Arthritis and Rheumatism* 20: 1231-1239 (1977), with monospecific antibodies directed against human rheumatoid synovial collagenase which detected high levels of immunoreactive collagenase at the sites of joint erosion (cartilagepannus junctions) but not in the cartilage of associated chondrocytes, and not in the synovium at sites remote from the resorbing front. Collagenases have also been demonstrated using many other different preparations derived from human rheumatoid joints and using tissues characterized by other types of arthritis such as osteoarthritis, Reiter's syndrome, pseudogout, juvenile rheumatoid arthritis, and scleroderma.

10 [0072] In periodontal disease affecting the tooth supporting apparatus, elevation of collagenolytic enzymes is evident, and destruction of collagen and connective tissue. See V.-J. Uitto, pp: 211-223 in *Proteinases in Inflammation and Tumor Invasion*, H. Tschesche, ed., Walter de Gruyter & Co., Berlin, N.Y. (1988).

15 [0073] Collagenases have been implicated in ulceration including corneal, epidermal, or gastric ulceration, Brown et al., *American J. of Ophthalmology* 72: 1139-1142 (1971), and, indeed, metalloproteinase inhibitors are used in the treatment of corneal ulceration, Slansky et al., *Annals of Ophthalmology* 2: 488-491 (1970).

20 [0074] In wound healing after surgery, TIMP-3 may have particular application for restenosis. Metalloproteinases contribute to the rearrangement of arterial cells, including blockage of the artery. Use of the present TIMP-3 may inhibit such arterial wall rearrangement. Delivery of antisense TIMP-3 nucleic acid may also provide benefit.

25 [0075] In the field of tumor invasion and metastasis, the metastatic potential of some particular tumors correlates with the increased ability to synthesize and secrete collagenases, Liotta et al., *Nature* 284: 67-68 (1980), and with the inability to synthesize and secrete significant amounts of a metalloproteinase inhibitor, Hicks et al., *Int. J. Cancer* 33: 835-844 (1984). These processes are related to the passage of tumor cells through connective tissue layers (basement membrane) from tissue sites to the circulation and vice versa, which could be retarded by TIMP-3. TIMP-3 similarly has therapeutic application in inhibiting tumor cell dissemination during removal of primary tumors, during chemotherapy and radiation therapy, during harvesting of contaminated bone marrow, and during shunting of carcinomatous ascites.

30 [0076] A limiting factor in the use of bone marrow transplantation for many advanced cancers with bone marrow involvement is the absence of adequate purging techniques. For example, metastatic interstitial pneumonitis following infusion of improperly purged bone marrow cells has been noted, Glorieux et al., *Cancer* 58: 2136-2139 (1986); Graeve et al., *Cancer* 62: 2125-2127 (1988). TIMP-3 administered during infusion of unpurged bone marrow cells will alleviate the need for developing expensive purging techniques.

35 [0077] Diagnostically, correlation between absence of TIMP-3 production in a tumor specimen and its metastatic potential is useful as a prognostic indicator as well as an indicator for possible prevention therapy.

40 [0078] Tumors may also become more or less encapsulated or fibrotic due to increased collagen deposition (or inhibition of breakdown) by both cancer cells and/or surrounding normal cells. Increased encapsulation promoted by TIMP-3 aids in clean tumor excision.

45 [0079] Other pathological conditions in which excessive collagen degradation may play a role and thus where TIMP-3 can be applied, include emphysema, Paget's disease of bone, osteoporosis, scleroderma, pressure atrophy of bone or tissues as in bedsores, cholesteatoma, and abnormal wound healing. TIMP-3 can additionally be applied as an adjunct to other wound healing promoters, e.g., to modulate the turnover of collagen during the healing process.

50 [0080] TIMP-3 also may have erythroid potentiating activity (i.e., stimulation of differentiation of early erythroid progenitors), and thus TIMP-3 may be useful in the treatment of various anemias.

[0081] In addition TIMP-3 may have application in the treatment of immunological disorders such as autoimmune diseases (e.g., rheumatoid arthritis, multiple sclerosis) based upon a potential ability to suppress B-cell differentiation as determined by the method of Pisko et al., *J. Immunol.* 136: 2141-2150 (1986).

55 [0082] Based on its ability to inhibit connective tissue degradation, TIMP-3 and/or other TIMP molecules have application in cases where inhibition of angiogenesis is useful, e.g., in preventing or retarding tumor development, and the prevention of the invasion of parasites. In addition, the present compositions and methods may be applicable for cosmetic purposes, in that localized inhibition of connective tissue breakdown may alter the appearance of tissue.

[0083] The present compositions and methods may also be useful in birth control or fertilization modulation as the TIMPs have been shown to prevent or retard follicular rupture, Branstrom et al., *Endocrinology* 122: 1715-1721 (1988), and interfere with embryo preimplantation development.

[0084] The present compositions and methods may be useful in the treatment of nerve cell disorders in that TIMP-3 may protect nerve cells from damage by preserving the basement membrane surrounding nerve cells.

Therefore, uses may involve BDNF, NT-3, NGF, CNTF, NDF, SCF, or other nerve cell growth or proliferation modulation factors.

5 [0085] As described above, the present TIMP-3 has wide application in a variety of disorders. Thus, another embodiment contemplated herein is a kit including the present polypeptides and optionally one or more of the additional compositions described above for the treatment of a disorder involving the degradation of extracellular matrix. An additional embodiment is an article of manufacture comprising a packaging material and a pharmaceutical agent within said packaging material, wherein said pharmaceutical agent contains the present polypeptide(s) and wherein said
10 packaging material comprises a label which indicates that said pharmaceutical agent may be used for an indication selected from the group consisting of: cancer, inflammation, arthritis, dystrophic epidermolysis bullosa, periodontal disease, ulceration, emphysema, bone disorders, scleroderma, wound healing, erythrocyte deficiencies, cosmetic tissue reconstruction, fertilization or embryo implant modulation, and nerve cell disorders. This article of manufacture may optionally include other compositions or label descriptions of other compositions.

15 [0086] The nucleic acids provided herein may also be embodied as part of a kit or article of manufacture. Contemplated is an article of manufacture comprising a packaging material and a pharmaceutical agent, wherein said pharmaceutical agent contains the presently provided nucleic acids and wherein said packaging material comprises a label which indicates that said pharmaceutical composition may be used for an indication benefiting from the modulation of said DNA expression, such as a gene therapy indication. Such gene therapy indications, as discussed above, include
20 the treatment of emphysema. A kit containing the nucleic acid(s) may include, optionally, additional factors affecting the ex vivo growth of recipient cells, such as SCF. See, e.g., Zsebo et al., PCT WO 91/05795.

25 [0087] A further embodiment of the invention is selective binding molecules, such as monoclonal antibodies specifically binding TIMP-3. The hybridoma technique described originally by Kohler and Milstein Eur. J. Immunol. 6, 511-519 (1976) has been widely applied to produce hybrid cell lines that secrete high levels of monoclonal antibodies against many specific antigens. Recombinant antibodies, (see Huse et al., Science 246: 1275 (1989)) may also be prepared. Such antibodies may be incorporated into a kit for diagnostic purposes, for example.

[0088] The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

30 EXAMPLE 1

Cloning and Expression of Human TIMP-3 cDNA

[0089] The overall cloning strategy involved two steps, the first, obtaining a fragment using PCR from a human fetal kidney cDNA library, and the second, using this partial clone to screen two different cDNA libraries for full length cDNA sequences.

35 [0090] Degenerate PCR primers derived from highly conserved regions of the TIMP gene family were used to amplify TIMP-3 cDNA from human fetal kidney cDNA. This product was then used as a probe to isolate clones from a human fetal kidney cDNA library and a normal human colonic mucosa cDNA library. Clones of 1240, 963 and 827 bp have
40 been isolated and sequenced. The longest clone encodes the entire 211 amino acid pro-polypeptide, having a mature polypeptide of 188 amino acids. The intermediate size clone is truncated but encodes the entire mature protein. The smallest clone is missing the region encoding the first 24 amino acids of the mature polypeptide. Also demonstrated is the expression and purification of mature polypeptide.

45 MATERIALS AND METHODS

Primers and Initial TIMP-3 DNA Source Used

[0091] Degenerate PCR primers were used in a first round screening of first strand cDNAs to obtain a partial TIMP-3 cDNA clone. The degenerate PCR primers were derived from highly conserved regions of the TIMP family of proteins were selected, (see Figure 4). They were also chosen because of the relatively low degeneracy of their codons.

50 [0092] The forward primer was derived from a sequence (VIRA) which is ubiquitous throughout the TIMP family and is found at positions 18-21 of the mature proteins. This 96-fold degenerate forward primer had 11 bases that encoded the TIMP sequence plus 6 bases for an *EcoRI* site and 2 extra bases (underlined)449-15: SEQ. ID No. 1: 5'-CGG AAT
55 TCG TNA THM GNG C-3'

[0093] A reverse primer corresponding to a region of ChIMP-3 (CIWTDM) was synthesized. This primer, 480-27, included a *BamHI* site and two extra bases (underlined):

SEQ. ID No. 2 5'-CGG GAT CCC ATR TCN GTC CAD ATR CA-3'.

[0094] An alternative reverse primer was also used:

5

SEQ. ID No. 3
480-28 CGG GAT CCR TCN GTC CAD ATR CA.

10 The corresponding region is somewhat variant. Amino acids 163-168 of ChIMP-3 are encoded by the version used here, and these were chosen because the M and I distinguished the ChIMP-3 from other TIMPs. It was not initially known if these differences would also be present in human TIMP-3 (if such TIMP did indeed exist), however, a bias away from TIMP-1 and TIMP-2 was used to limit unwanted amplifications. The M at position 168 was especially useful.
15 As a result of its location at the 5' end of the reverse primer, it would not interfere with the PCR process if there were mismatches and it would favor TIMP-3 amplification over other DNAs if this choice were correct.

Amplification of First Strand cDNAs Using Primers

20 [0095] First, the degenerate primers were used to amplify PCR products from the two first strand cDNAs. After a second round of amplification the PCR products of these were subcloned, and one was selected which was used as a probe for cDNA libraries, as described below.

25 [0096] Oligonucleotide synthesis. Oligonucleotides were synthesized on Applied Biosystems 394 automated synthesizers using standard phosphoramidite chemistry. Degenerate oligonucleotides, which were synthesized in greater than 200 nmole quantities, were purified by butanol extraction. Nondegenerate oligonucleotide were synthesized in smaller amounts and were purified Tritylon using Poly-pak (Glen Research Corp., Sterling, VA) cartridges following the manufacturer's instructions. Trityl-off purification was done using 1 × 25 cm Sephadex G-50 chromatography columns and TEAB as the elution buffer.

30 [0097] Polymerase Chain Reaction. All PCR was performed on Perkin Elmer model 9600 instruments using Perkin Elmer Cetus (Norwalk, CT) GeneAmp kits according to the manufacturer's instructions which are herein incorporated by reference.

35 [0098] The first round of PCR consisted of 5 cycles at 94°C for 20 seconds, 50°C for 20 seconds and 72°C for 30 seconds. This was followed by 30 cycles at 94°C for 20 seconds, 50°C for 20 seconds and 72°C for 30 seconds. The PCR products were run on a 2% agarose (SeaKem GTG, FMC, Rockland, ME) gel, prestained with ethidium bromide (Sigma, St. Louis, MO), and the bands in the predicted size range were punched out of the gel using a Pasteur pipette.

40 [0099] The PCR products were then re-amplified directly from the gel fragments using the same PCR primers and the following program: 1 cycle of 5 minutes at 95°C followed by 25 cycles of 94°C for 20 seconds, 50°C for 20 seconds, and 72°C for 30 seconds. This process was performed a second time in an effort to obtain large quantities of relatively pure material for subcloning and restriction analysis.

45 [0100] Purification and Subcloning of PCR Products. The PCR products were run through Centricon-100 columns (Amicon, Beverly, MA) to facilitate the DNA to be cleaved with restriction endonucleases. The DNA was then cut with *EcoRI* and *BamHI* to ensure that they would not be internally cleaved during the subcloning process. PCR products were cloned into pUC19 after treatment with proteinase K (Crowe et al., 1991) to enhance the cloning efficiency. Colonies were rapidly screened by PCR amplification with vector primers 382-3 SEQ. ID No. 4 (5'-GTT TTC CCA GTC ACG ACG-3') and 382-4 SEQ. ID No. 5 (5'-GAA TTG TGA GCG GAT AAC-3'). These products were purified using Centricon-100 concentrators and were sequenced.

50 [0101] Results. As shown in Figure 2 three bands resulted from amplification with the degenerate primers. Cloned DNA from two of the bands was sequenced; the third band could not be purified sufficiently to allow subcloning and sequencing.
55 [0102] The smaller of the two sequenced bands was the desired 402 bp fragment and the larger band presumably resulted from false priming to the region encoding CSWYRG (amino acids 169-174 of the mature polypeptide of Figure 1) and was 489 bp. The 402 bp fragment corresponds to the nucleic acid encoding the region encompassing Vallle-ArgAla(Lys) to CysLeuTrpThrAspMet of Figure 1, with an *EcoRI* on the 5' side, and an *BamHI* on the 3' side. Also, the codon for isoleucine on the 3' end is replaced with the codon for leucine.

cDNA library screeningScreening of a first cDNA library.

5 [0103] Library. The first library screened was an the oligo(dT)-primed λgt11 Clontech human 20 and 24 week fetal kidney cDNA library (Clontech).

10 [0104] Probes. The first round of cDNA screening was done with the insert of one of the cloned degenerate PCR products previously described, the 402 bp insert. A low level of background was observed as a result of contamination with pUC19 vector DNA. Consequently, the phage supernatant from a partially purified λgt11 clone obtained from the first round of cDNA screening was used as a PCR template. Friedman et al., Nucl. Acids Res. 17: 8718 (1988). This provided a probe of high quality and purity. The Primer 495-21, SEQ. ID No. 6 5'-CGG AAT TCT GGT CTA CAC CAT CAA GC-3' corresponded approximately to the YTIK domain and including an EcoRI site and two additional bases. Primer 496-16, SEQ. ID No. 7 5'-CAT GTC GGT CCA GAG ACA CTC G-3', corresponded to the CLWTDM region and did not include any restriction sites. This resulted in a 333 bp fragment. The sequence of the 333 bp fragment was a portion of the 402 bp fragment sequence. The 333 bp fragment was used as a probe for all of the northern blot analyses and for all further cDNA library screening. The 333 bp fragment corresponds to the region of Figure 1 encoding TyrThrIle-Lys through CysLeuTrpThrAspMet and the EcoRI site mentioned above.

15 [0105] Plaque Hybridization About 200,000 phage were plated on ten 150 mm plates, lifted in duplicate onto Schleicher & Schuell supported nitrocellulose membranes and probed with a randomly primed, ³²P-labeled (Stratagene) 20 402 bp fragment described above. Prehybridizations and hybridizations were performed overnight at 42°C using the following reagents (for 50 ml of solution):

25	12.5 ml	20X SSPE
	5 ml	0.5 N NaHPO ₄ pH 6.8
	0.1 ml	0.50 M EDTA pH 8.0
	25 ml	formamide
	2.5 ml	50X Denhardt's
	0.25 ml	20% SDS
30	0.5 ml	10 mg/ml tRNA (calf's liver)
	1 ml	10 mg/ml salmon sperm DNA (not used in the pre-hybridization solution)
	4.15 ml	H ₂ O (3.15 ml used in the hybridization solution)

35 [0106] The filters were washed in 0.25 X SSC at 42°C. Two positively hybridizing plaques were purified, resulting in 2 independent clones here named Timp3clone7 and Timp3clone2. DNA from bacteriophage lambda was purified using a Qiagen Lambda DNA purification kit (Chatsworth, CA). Plate lysates from 10 confluent 135 mm petri dishes were pooled for each specimen. 300 µl of a solution containing 20 mg/ml RNase, 6 mg/ml DNase I, 0.2 mg/ml BSA, 10 mM EDTA, 100 mM Tris-HCl, 300 mM NaCl, pH 7.5 were added and incubated at 37°C for 30 minutes. 10 ml of ice cold 30% polyethylene glycol (PEG 6000), 3 M NaCl were mixed in and incubated on ice for 60 minutes.

40 [0107] After centrifugation at 10,000 × g for 10 minutes, the supernatant was discarded. The pellet was resuspended in 10 ml of a solution containing 100 mM Tris-HCl, 100 mM NaCl and 25 mM EDTA, pH 7.5. 10 ml of a solution containing 4% SDS was gently added and the mixture was heated at 70°C for 10 minutes and then cooled on ice. 10 ml of 2.55 M potassium acetate, pH 4.8 was mixed in quickly and the solution was centrifuged at 4°C at 15,000 × g for 30 minutes. The supernatant was run on a Qiagen tip-500 column which had been equilibrated with 10 ml of 750 mM NaCl, 50 mM MOPS, 15% ethanol, pH 7.0. The column was then washed with 30 ml 1.0 M NaCl, 50 mM MOPS, 15% ethanol, pH 7.0. Finally, the column was eluted with 15 ml of 1.25 M NaCl, 50 mM MOPS, 15% ethanol, pH 8.2. The eluate was precipitated in 0.7 volumes of isopropanol and centrifuged at 4°C for 30 minutes. The pellet was air dried for 5 minutes and cut with Boehringer Mannheim (Mannheim, Germany) high concentration EcoRI.

50 [0108] The inserts which had hybridized to the 333 bp probe were purified from agarose gel slices using a Qiaex DNA extraction kit (Qiagen, Chatsworth, CA). A solution of 3 M NaI, 4 M NaClO₄, 5 mM Tris-H, pH 7.5 at three times the volume of the gel slice was added, along with 0.1 times the gel slice volume of 1 M mannitol and 10 ml of Qiaex resin in a 1.5 ml microcentrifuge tube. This mixture was heated at 50°C for 10 minutes or until the agarose is completely dissolved. The DNA was allowed to adsorb at room temperature for 5 minutes and then the tubes were briefly centrifuged (6 seconds). After the supernatants were discarded, the Qiaex resin in the tubes were washed in a solution containing 8 M NaClO₄, and centrifuged (6 seconds). This wash and centrifugation was repeated and was followed by 2 washes (each followed by 6-second centrifugations) in a solution containing 70% ethanol, 100 mM NaCl, 10 mM Tris-HCl, 1 mM EDTA, pH 7.5. The resin was air dried and eluted in 20 µl of water.

[0109] The purified inserts were cloned into pUC19 (New England Biolabs) using Boeringer Mannheim's T4 DNA polymerase. There was an insert to vector (molar) ratio of approximately 5:1. Ligations were performed overnight at 14°C. The ligated material was ethanol precipitated in the presence of glycogen to increase the recovery. This material was then electroporated into BRL's (Gibco-BRL, Gathersburg, MD) electroporation competent DH10B cells.

5 [0110] Preparations of plasmid DNA were made using using Qiagen plasmid DNA purification kit. A 10 ml overnight culture of a single bacterial colony was grown in terrific broth [Tartoff and Hobbs, Bethesda Res. Lab. Focus 9:12 (1987). Per liter: 12 g bacto-tryptone, 24 g bacto-yeast extract, 4 ml glycerol] with 50 µg/ml ampicillin. The overnight growth was used to inoculate a 250 ml culture in a sterile 1-liter baffled flask containing terrific broth with 50 µg/ml ampicillin. After this grew to saturation, the medium was centrifuged at 5000 rpm for 10 minutes. The bacterial pellet was resuspended in 10 ml of 100 µg/ml RNaseA, 50 mM Tris-HCl. 10 ml of 200 mM NaOH, 1% SDS was added to the resuspended pellet and the mixture was incubated at room temperature for 5 minutes. 10 ml of 2.55 M KAc, pH 4.8 was added and mixed gently. The material was immediately centrifuged at 10000 rpm for 10 minutes. The supernatant was filtered through a cotton gauze pad and the lysate that was particle-free was added to a Qiagen tip-500 column following the same procedure as per the lambda DNA preparation procedure.

10 [0111] Screening of a second cDNA library. A CDNA library from human colonic mucosa, kindly provided by Jim Pipas of the University of Pittsburgh, was the second library screened for TIMP-3 cDNA. This library used Uni-Zap (Stratagene, La Jolla, CA) as the vector and had a titer of 2.4×10^{10} pfu/ml. Hybridization was performed as with the kidney library, using the 333 bp probe. The Uni-Zap vector has a pBluescript phagemid which was excised from the phage to which the probes hybridized, and sequenced directly.

15 [0112] Phage particles were isolated and amplified as follows. Phage particles were released into the SM buffer by incubating for 2 hours at room temperature. In a 50 ml test tube, 200 µl of O.D.₆₀₀=1.0 XL1-Blue cells and 200 µl of the lambda Zap phage were combined with 1 ml of R408 helper phage which had a titer of 10^{10} pfu/ml. This mixture was incubated at 37°C for 15 minutes. 3 ml of 2xYT medium (per liter: 16 g bacto-tryptone, 10 g bacto-yeast extract, 5 g NaCl) were added and the mixture was then incubated for 2.5 hours at 37°C with shaking. The tube was heated

20 at 70°C for 20 minutes and then centrifuged at $4000 \times g$ for 5 minutes.

25 [0113] To rescue the phagemid, 50 µl of the heat-disrupted phage stock were incubated with 200 µl of O.D.₆₀₀=1.0 XL1-Blue cells in a 1.5 ml tube. Additionally, 10 µl of a 10^{-2} dilution of heat-disrupted phage were incubated with 200 µl of O.D.₆₀₀=1.0 XL1-Blue cells in a separate 1.5 ml tube. The tubes were incubated at 37°C for 15 minutes and the cells were then plated on LB ampicillin plates and incubated overnight at 37°C. Colonies appearing on the plate contained the pBluescript SK- double stranded phagemid with the cloned DNA insert.

30 [0114] This screening resulted in one clone, here named "TIMP3HCM3," (see Figure 16), lacking a portion encoding the N-terminus of the mature polypeptide.

DNA Sequencing

35 [0115] All sequencing was performed on Applied Biosystems, Inc. (ABI) 373A Automated Sequencers. PCR products were sequenced using nested pUC vector dye-primers and ABI's catalyst to perform the reactions.

40 [0116] Double stranded cDNAs cloned into pUC19 were sequenced using ABI's Prism Ready Reaction Dye-Deoxy Terminator Cycle Sequencing Kit using the protocol that came with the kit. For areas of high GC content leading to hairpin loops, reactions were done with the following changes from the standard kit protocol: denaturation at 98°C for 30 seconds, 12 U Amplitaq, substitution of New England Biolabs (NEB) Vent Polymerase buffer for the ABI TACS buffer and, 30 cycles instead of 25 cycles.

Sequent Analysis

45 [0117] DNA and deduced amino acid analyses used the Genetics Computer Group (GCG) sequence analysis software package from the University of Wisconsin Department of Genetics, Genetic Computer Group, Inc., University Research Park, 575 Science Drive, Suite B, Madison, Wisconsin 53711.

Expression of Recombinant Human TIMP-3 in E. coli

50 [0118] The coding sequence of Timp3clone7 was amplified by PCR using standard kit protocol. Primer 544-29 SEQ. ID No.8 (5'-AAC AAA CAT ATG TGC ACA TGC TCG CCC AGC C-3') consists of nucleotides 351 to 369, which encodes TIMP-3 amino acids 24-29 (1-6 of the mature protein of Figure 1). An *NdeI* site and 6 extra bases (underlined) were included to facilitate subcloning into a bacterial expression vector. The methionine initiator codon, (italics), was added to facilitate expression. The downstream primer, 532-13, SEQ. ID No.9 (5'-CGG GAT CCT ATT AGG GGT CTG TGG CAT TGA TG-3') corresponds to nucleotides 896 to 914 (of Figure 1) with an added *BamHI* site and 2 additional bases (underlined) as well as two stop codons (italicized). The naturally occurring stop codon, TGA (TCA on the reverse

complement) was changed to TAA (TTA on the reverse complement), since it is a more efficient stop in *E. coli*. The second stop codon, TAG, (CTA on the reverse complement) was added as a backup.

[0119] The vector pCFM3102, as described below, was digested with *NdeI* and *BamHI* overnight as was the 589 bp PCR fragment encoding TIMP-3. The reaction was stopped by extraction with phenol/chloroform followed by extraction with chloroform alone. The aqueous layer was then passed through a 1 ml Sephadex G-50 spin column (in a 1 ml syringe) that was equilibrated with 200 μ l 10 mM Tris-HCl, 1 mM EDTA pH 8.0. The flow-through from the column was collected and precipitated with 0.1 volumes of 3 M NaAc, pH 5.4 and 2.5 volumes of 100% ethanol. After centrifugation, the pellet was washed in 70% ethanol and dried in a Speed-Vac (Savant). The pellets were resuspended in 20 μ l Super-Q water.

[0120] A mock ligation containing cut pCFM3102 with no insert was done in addition the TIMP-3::pCFM3102 ligation. Ligations were performed overnight at 14°C, using Boehringer Mannheim T4 DNA ligase. They were then precipitated, washed and dried as above. The pellets were then resuspended in 5 μ l of Super-Q water. 2.5 μ l of each ligation was used to electroporate 40 μ l of electroporation competent cells.

[0121] Electroporation of plasmid into *E. coli* occurred in 0.1 cm cuvettes (Bio-Rad) at 1.9 kV, 200 ohms, 25 μ F using a Bio-Rad Gen Pulser and with immediate recovery in 5 ml of SOC medium. The cells recovered at 28°C for 11.3 hours and were plated out onto LB plates containing kanamycin. The plates were incubated at 28°C overnight. Colonies were screened for inserts by PCR using vector-specific primers 315-21 SEQ. ID No. 10 (5'-ACC ACT GGC GGT GAT ACT GAG-3') and 315-22 SEQ. ID No. 11 (5'-GGT CAT TAC TGG ACC GGA TC-3'). Colonies having inserts gave PCR products that are 589 bp larger than the PCR product derived from the original vector without an insert.

Construction of expression plasmid pCFM3102

[0122] Expression of the mature protein was accomplished in *E. coli* using a plasmid vector. A culture of this *E. coli*, containing plasmid encoding a mature polypeptide as presented in Figure 1, is deposited at the ATCC, accession no. 69454.

[0123] The plasmid used was derived from pCFM836, which is fully described in U.S. Patent No. 4,710,473. The construction for the present plasmid (denominated pCFM3102) from the described pCFM836 plasmid (U.S. Patent No. 4,710,473) was by destroying the two endogenous *NdeI* restriction sites, by end filling with T4 polymerase enzyme followed by blunt end ligation, by replacing the DNA sequence between the unique *AatII* and *ClaI* restriction sites containing the synthetic P_L promoter with a similar fragment obtained from pCFM636 (Patent No. 4,710,473) containing the P_L promoter, by substituting the small DNA sequence between the unique *ClaI* and *KpnI* restriction sites with an oligonucleotide containing a number of restriction sites, and by making a series of site directed base changes by PCR overlapping oligonucleotide mutagenesis through the intermediate pCFM1656 vector (4799 base pair).

Fermentation

[0124] The inoculum for the fermentation was started by transferring 0.1 ml of a glycerol stock at 1 O.D./ml in LB + 17% glycerol of ATCC Accession No. 69455 (*E. coli* host cells containing the pCFM3102 with inserted TIMP-3 coding sequences) into a 2-L nippled flask containing 500 ml of Luria Broth (10 g/L Trypticase-Peptone, 10 g/L yeast extract, and 5 g/L sodium chloride). The culture was placed in a shaking platform incubator at 30°C for 16 hours at 250 rpm. The culture was then transferred to 8 liters of sterile medium in a BioLafitte 15-L fermentor.

[0125] The 8 liters of medium that were sterilized in place in the fermentor consisted of the following:

45	10 g/L 5.25 g/L 3.5 g/L 4.0 g/L 1.25 g/L	yeast extract ammonium sulfate dibasic potassium phosphate monobasic potassium phosphate sodium chloride
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[0126] After the sterilized medium cooled to 30°C the following was added:

55	40 g 8 g 16 ml	glucose magnesium sulfate-heptahydrate trace metals solution ¹
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[0127] The pH of the medium was then adjusted to 7.0 using concentrated phosphoric acid. The other parameters

of the fermentation during this batch phase were set as follows:

air flow rate = 31.0 L/min

agitation = 350 rpm

5 dissolved oxygen readout set at 60%
oxygen flow rate = 0
back pressure = 0.5 bar

[0128] Once the culture in the fermentation vessel reached at O.D.600 of 6.0, a concentrated solution of glucose
10 and organic nitrogen was started using a schedule that ramps the feed flow according to the O.D. of the culture. This concentrated feed (Feed 1) consisted of the following:

15	50 g/L	Trypticase-peptone
	50 g/L	yeast extract
	450 g/L	glucose
	8.5 g/L	Magnesium-heptahydrate
	10 ml	trace metals solution ¹
20	10 ml	vitamin solution ²

[0129] At the time that the concentrated feed was first introduced into the fermentor, the following changes were made:

25 agitation raised to 850 rpm

back pressure raised to 0.8 bar

[0130] Using the concentrated feed, the O.D. was increased to 30. At that point the culture was induced by raising the temperature to 42°C. Other changes were made as follows:

30 air flow rate decreased to 24 L/ min
oxygen flow rate increased to 3 L/min
feed 1 decreased to 0
feed 2 started at 300 ml/hr

35 Feed 2 consisted of the following:

200 g/L Trypticase-peptone

100 g/L yeast extract

110 g/L glucose

40 [0131] After 4 hours at 42°C the fermentation was halted and the cells were harvested by centrifugation into plastic bags contained within a one liter centrifuge bottle. Centrifugation was at 400 rpm for 60 minutes. At the end of this period, the supernatant was removed and the remaining cell paste was frozen at -90°C.

¹ Trace metals solution:	
27 g/L	FeCl ₃ ·6H ₂ O
2 g/L	ZnCl ₂ ·4H ₂ O
2 g/L	CaCl ₂ ·6H ₂ O
2 g/L	Na ₂ ·MoO ₄ ·2H ₂ O
1.9 g/L	CuSO ₄ ·5H ₂ O
0.5 g/L	H ₃ BO ₃
100 ml/L	concentrated HCl

² Vitamin solution:	
0.42 g/L	riboflavin
5.4 g/L	pantothenic acid
6 g/L	niacin
1.4 g/L	pyridoxine hydrochloride
0.06 g/L	biotin
0.04 g/L	folic acid

10 NH₂-terminal amino acid sequencing

[0132] NH₂-terminal amino acid sequence of *E. coli*-derived recombinant TIMP-3 protein was determined to be identical to the sequence deduced from the cDNA clones. The methionine initiator from the construct was cleaved off. There
 15 was no other detected proteolytic processing at the TIMP-3 NH₂-terminus. No assignment was made for cys-1 and cys-2 since the protein sample was reduced and reduced cysteines cannot readily be detected by this method. Therefore, the sequence read as follows: X-T-X-S-P-S-H-P-Q-D-A-F-

Methods

[0133] Partially purified recombinant TIMP-3 present in *E. coli* inclusion bodies was electrophoresed on an SDS polyacrylamide gel and electroblotted onto a PVDF membrane for sequence analysis. NH₂-terminal amino acid analysis was performed on a gas-phase sequenator (model 477, Applied Biosystems, Foster City, CA) according to published protocols. Hewick et al., J. Biol. Chem., 256: 2814-2818 (1981). The sequenator was equipped with an on-line phenylthiohydantoin (PTH) amino acid analyzer and a model 900 data analysis system (Hunkapiller et al., Methods of Protein Microcharacterization, Clifton, NJ: pp. 223-247 (1986)). The PTH-amino acid analysis was performed with a micro liquid chromatography system (model 120) using dual syringe pumps and reversed phase (C-18) narrow bore columns (Applied Biosystems, Inc.), with the dimensions of 2.1 mm × 240 mm.

30 Protein Purification

[0134] Approximately 435 g wet weight of *E. coli* cell paste, harvested from the fermentation run was resuspended to a volume of 1760 ml in water and broken by two passes through a microfluidizer. The cell lysate was centrifuged at 17,700 × g for 30 min, and the pellet fraction was washed once with water (by resuspension and by recentrifugation). A portion of the washed pellet material (3.1% of the total) was resuspended in 10 ml of 50 mM Tris-HCl/50 mM dithiothreitol/2% (w/v) Sodium N-lauroylsarcosine, pH 8.5. After incubation at 50°C for 5 min, and at room temperature for 3 hr, the mixture was centrifuged at 20,000 × g for 60 min. The supernatant was applied to a Sephadryl S-200 gel filtration column (Pharmacia; 2 × 23 cm) equilibrated in 20 mM Tris-HCl/1% sodium N-lauroylsarcosine, pH 8.0, at room temperature.

Fractions of 1 ml were collected at a flow rate of 5 ml/hr and analyzed by A₂₈₀ and by SDS/polyacrylamide gel electrophoresis (PAGE). Fractions 43-53 were pooled, and the pool was dialyzed over a 3-day period against 20 mM Tris-HCl (pH 8.0), 0.02 % (w/v) sodium azide, at 4°C.

[0135] Figure 3 presents a silver stained SDS-PAGE gel of the partially purified expression product from this fermentation. Lanes 4 and 5 contain reduced *E. coli* derived TIMP-3, pre- and post-dialysis. Lanes 9 and 10 contain unreduced *E. coli* derived TIMP-3, pre- and post-dialysis. As can be seen, the apparent molecular weight for reduced material is approximately 22kDa.

[0136] As can be seen from Figure 3, the post-dialysis yield was reduced; the polypeptide appeared to be somewhat unamenable to solubilization. In the present process, the presence of inclusion bodies containing relatively insoluble material resulted in a reduced yield of purified and isolated TIMP-3. Although this resulted in a partially purified product, one skilled in the art will recognize methods to obtain a purified and isolated polypeptide. For example, one may use different detergents as solubilizing agents, or use a different expression system, for example, one which permits secretion of the polypeptide (and thus elimination of inclusion bodies).

[0137] Expression and purification was also attempted using eucaryotic cells (COS-7 cells), however no active recombinant TIMP-3 polypeptide was observed. This may have been due to adherence of the recombinant TIMP-3 polypeptide to extracellular matrix material produced by COS-7 cells. One possible way to obtain active protein from a mammalian host cell may be to use cells which are non-adherent, and therefore produce no significant amount of extracellular matrix material. The recombinant polypeptide would then be found in the conditioned culture medium. For example Jurkat cells or U937 cells may be used for recombinant polypeptide expression, and other non-adherent host

cells and expression systems will be apparent to those skilled in the art.

Results of Screening Two cDNA Libraries and Expression of Recombinant Human TIMP-3

5 [0138] The work herein presents the cloning and expression of a third class of mammalian TIMP family members, herein collectively referred to as "TIMP-3". The nucleotide sequence obtained from a human fetal kidney cDNA library is presented in Figure 1. Seq. ID No. 12 As can be seen, the nucleic acid sequence obtained contains 1240 base pairs. The predicted amino acid sequence is also presented. Seq. ID No. 13 (The amino acid sequence is predicted, as the polypeptide itself was not fully sequenced. One skilled in the art may sequence the expression product of the *E. coli* deposited at the ATCC, accession no. 69455). The predicted initial cysteine of the mature protein is number +1. This prediction is based upon comparison to other members of the TIMP family.

10 [0139] Figure 4 presents this comparison among the known members of the TIMP family. Bullet points (•) indicate those amino acid residue which are unique to the TIMP-3 of Figure 1 obtained from expression of human cDNA, and bold-face type indicates conserved residues.

15 [0140] As can be see, the present human recombinant TIMP-3 of Figure 1 is distinct from all other members of the TIMP family. While possessing the conserved cysteine residues and other conserved amino acids within the family (39, total), at least 23 amino acid residues are unique to the illustrated human recombinant TIMP-3.

20 [0141] Figures 5-13 illustrate the differences between the present human recombinant TIMP-3 of Figure 1 and chicken TIMP-3 ("ChIMP-3," Figures 5-7), human TIMP-2 (Figures 8-10), and human TIMP-1 (Figures 11-13), at both the amino acid and nucleic acid levels. The Figures contain a solid line between amino acid residues which are identical, and dots indicating the degree of evolutionary distance. (For Figures 5, 8, and 11, illustrating amino acid alignment, the numbering at position "1" is for the mature polypeptide.)

25 [0142] At the amino acid level, TIMP-3 and ChIMP-3 are approximately 80% identical, with identical amino acids being more or less dispersed discontinuously, (Figure 5). Figure 6 shows that, at the nucleic acid level, Figure 1 TIMP-3 DNA is approximately 74% homologous with ChIMP-3 DNA, between nucleic acids 151-1087 (TIMP-3) and 1-886 (ChIMP-3). Figure 7 shows that even analyzing the region of maximal homology, base pairs 282-1040 from Figure 1 TIMP-3, and 113-884 for ChIMP-3), there is approximately 78% identity.

30 [0143] Figures 8-10 illustrate a comparison between human recombinant TIMP-3 of Figure 1 and human TIMP-2. At both the amino acid level and the nucleic acid level, there are greater distinctions than with ChIMP-3. Figure 8 shows that there is approximately 46% identity at the amino acid level. Figure 9 shows that, at the nucleic acid level, the overall homology is approximately 52% overall, and approximately 60% in the region of maximal homology (Figure 10).

35 [0144] Figures 11-13 illustrate a comparison between human recombinant TIMP-3 of Figure 1 and human TIMP-1. At the amino acid level, there is approximately 39% identity (Figure 11), and approximately 47% overall homology at the nucleic acid level. There is approximately 65% identity in the region of maximal homology.

40 [0145] Biochemically, the calculated isoelectric points (pI) of the mature TIMP-3 polypeptide and its pre-cursor are 9.16 and 8.80, respectively. There is a potential glycosylation site at the carboxy-terminal sequence (184:NAT). While naturally occurring ChIMP-3 is reported to be non-glycosylated (Pavloff et al., *supra*, J. Biol. Chem. 267: at 17323), it is not currently known whether naturally occurring human TIMP-3 is glycosylated. Regardless, the present invention includes polypeptides with additional chemical moieties, such as carbohydrates. The hydrophobic leader of the Figure 1 material is 23 amino acids long. Sequencing of the N-terminus confirmed the identity of the first 12 amino acids of the mature recombinant polypeptide.

45 [0146] The cloning and expression described herein demonstrates that the present TIMP-3 polypeptides represent new members in the TIMP family.

45 EXAMPLE 2

Expression of TIMP-3 In Various Cell Types

50 [0147] A variety of cells were tested for the expression of TIMP-3 RNA (which would indicate polypeptide expression). The results show that among normal (i.e., non-cancerous) cells, expression is observed in cells associated with extracellular matrix activity (i.e., growth or degradation). The normal cells (or tissues) where TIMP-3 RNA expression was seen (Figures 14A and B) are placenta, stromal cells, embryonic lung, newborn foreskin (one of two samples being slightly positive), and (slightly positive) adult lung. Among the cancer cells tested, some were positive, some were negative. For example, various breast adenocarcinoma cell lines yielded disparate results; with one was positive, one was negative, one was slightly positive. This may indicate temporal expression, in that TIMP-3 expression may vary over the course of disease progression, although the significance is unclear. Table 2, below, presents a description of the cells tested and the results. Below are the methods.

55 [0148] In many of the positive cell lines three mRNA bands of approximate 2.2, 2.5 and 4.4 kb size were detected.

The significance of the different mRNA bands is unknown but may represent alternative splicing or extended 3' or 5' untranslated regions. These may be RNAs encoding different naturally occurring variants.

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TABLE 2
ATCC Numbers Plus Description

<u>ATCC cell line</u>	<u>ATCC Number</u>	<u>Description</u>	<u>Poly A northern</u>	<u>Total RNA northern</u>
Hs 294T	HTB 140	metastatic melanoma	strongly positive	strongly positive
HepG2	HB 8065	hepatocellular carcinoma	slightly positive	slightly positive
A-704 plus or minus	HTB 45	adenocarcinoma, kidney	negative	negative
HUT 78	TIB 161	T cell lymphoma	negative	negative
MCF-7 plus	HTB 22	breast adenocarcinoma	slightly positive	slightly positive
MCF-7 minus	HTB 22	breast adenocarcinoma	negative	negative
MDA-MB-231	HTB 26	breast adenocarcinoma	positive	positive
MDA-MB-453	CRL 131	breast carcinoma	negative	negative
Hs 68	CRL 1635	newborn human foreskin	slightly positive	slightly positive
Hs 27	CRL 1624	newborn human foreskin	negative	negative
A-172	CRL 1620	glioblastoma	strongly positive	strongly positive
Hs 578 T	HTB 126	ductal carcinoma, breast	borderline positive	borderline positive
A-498	HTB 44	carcinoma, kidney		
293	CRL 1573	transformed embryonal kidney		
SK-NEP-1	HTB 48	Wilms' tumor (kidney)	borderline positive	borderline positive
WI-38	CCL 75	normal embryonic lung	borderline positive	borderline positive
WI-26 VA4	CCL 95.1	SV40 virus transformed lung	borderline positive	borderline positive
CCD-11Lu	CCL 202	normal lung		
DU 4475	HTB 123	breast carcinoma, metastatic nodule		
BT-474	HTB 20	ductal carcinoma, breast	negative	negative
Caov-3	HTB 75	adenocarcinoma, ovary	slightly positive	slightly positive
SK-OV-3	HTB 77	adenocarcinoma, ovary	negative	negative
SK-Hep-1	HTB 52	adenocarcinoma, liver	slightly positive	slightly positive

Methods

[0149] Two types of Northern blots were performed, one on total RNA transcripts, and one using poly A+ tailed

transcripts.

[0150] Total RNA Preparation. Total RNA for the total RNA northern was extracted from cells using a modification of a published protocol (Chomczynski and Sacchi, Anal. Biochem. 162: 156-159 (1987).

[0151] Cells grown in $2 \times 10\text{cm}$ petri dishes (approximately 2×10^6 cells), were washed two times with cold 1x PBS.

5 After all of the PBS was aspirated off, 500 μl of an aqueous solution containing the following was added to each dish: 4 M guanidinium thiocyanate (Fluka), 25 mM sodium citrate pH 7.0 (Mallinckrodt), 0.5% sarcosyl (Sigma, St. Louis, MO) 0.1M β -mercaptoethanol (Sigma, St. Louis, MO). The cell lysate was pipetted into a 1.5 ml Eppendorf microfuge tube and was sheared with a 25 gauge needle.

10 [0152] Sodium acetate (pH 4) was added to the 500 μl lysate to make a final concentration of 0.2 M. The mixture was shaken vigorously by hand. 1/5 volume of chloroform was added and mixed thoroughly. The tubes were spun at 15,000 rpm for 5 minutes at 20°C in a Tomy MTX-100 centrifuge. The tubes were inverted to allow the white precipitate layer to separate from the aqueous layer instead of respinning. The RNA was re-extracted with phenol and chloroform two additional times and was extracted one final time with chloroform. 1 ml of isopropanol was added to the microfuge tube and the mixture was precipitated at -20°C overnight. The next day it was spun at 15,000 rpm for 15 minutes. The 15 pellet was washed with 1 volume of 80% ethanol, re-spun, and dried in a Speed Vac (Savant, Farmingdale, NY).

[0153] The pellet was resuspended in 400 μl of the guanidinium solution which contained β -mercaptoethanol (Sigma, St. Louis, MO). 800 μl of ethanol was added to this mixture, which was then spun at 15,000 rpm for 15 minutes and washed with 80% ethanol. This pellet was resuspended in 20 μl of water and the O.D. was determined.

20 [0154] Poly A+ RNA Preparation. Poly A+ RNA was prepared using Clontech (Palo Alto, CA) oligo dT-cellulose spun columns. 2×1 ml of a high salt buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA, 0.5 M NaCl) was washed through the columns and drained by gravity. Total RNA, isolated as described above, was resuspended in 1 ml of elution buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA, 3M NaCl) and was heated at 68°C for 3 minutes. 0.2 ml of sample buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA, 3M NaCl) was added to the RNA solution, which was then placed on ice.

25 [0155] The samples were loaded onto the freshly equilibrated columns and allowed to soak under gravity. The columns were placed inside 50 ml tubes and were centrifuged at $350 \times g$ for 2 minutes. The eluates were discarded. 0.25 ml of the high salt buffer (see above) was added to each column and the columns were centrifuged at $350 \times g$ for 2 minutes. This wash was repeated once. In each case, the eluates were discarded. The columns were then washed 3 times with low salt buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA, 0.1 M NaCl) and centrifuged each time at $350 \times g$ for 2 minutes. The eluates were discarded in each instance. Sterile 1.5 ml microcentrifuge tubes were placed inside 30 of the 50 ml tubes to collect subsequent elutions. 0.25 ml of elution buffer (10 mM Tris-HCl [pH 7.4], 1 mM EDTA,) warmed to 65°C were applied to the columns, which were then spun at $350 \times g$ for 2 minutes. This procedure was repeated 3 times for a total of 4 elutions per column. For each column, all of the elutions were collected in a microcentrifuge tube. The eluents were ethanol precipitated as above.

35 [0156] Northern Blotting. 10 μg of total RNA was loaded in each lane. The sample buffer included 10 μl of formamide, 3.5 μl of formaldehyde, 2 μl of 10x MOPS, 2 μl of loading dye, 0.2 μl of ethidium bromide, and 6.5 μl of RNA sample in water. The poly A+ RNA blot had 3 μg of mRNA loaded in each lane.

40 [0157] The gels for the northern blots consisted of 1.5 g of agarose melted in 95 ml of water and then cooled to 60°C. 30 ml of 5x MOPS and 25 ml of formaldehyde (pH 4.7) were added to the cooling agarose solution. Prior to transfer, the gels were trimmed to remove excess gel. They were then soaked in distilled water for 5 minutes, followed by a 10 minute soak in 50 mM NaOH, 10 mM NaCl at room temperature. The gels were neutralized in 0.1 M Tris-HCl, pH 7.5 for 45 minutes and then soaked in 20X SSC for 1 hour. Transfer occurred overnight in 10X SSC. The gels were blotted onto Schleicher & Scheull (Keene, NH) nitrocellulose membranes. The total RNA gel was blotted onto pure nitrocellulose and fixed by UV crosslinking using a Stratalink (Stratagene, La Jolla, CA). The poly A+ gel was blotted onto supported nitrocellulose and was baked in a vacuum oven for 2 hours at 80°C.

45 [0158] The blots were hybridized in a manner similar to the screening of the cDNA library. The sole difference is that for the northern blot analysis, RNase-free reagents were used wherever possible.

EXAMPLE 3

50 In Vitro Activity of Human Recombinant TIMP-3

Modified Zymogram

55 [0159] DeClerck et al. J. Biol. Chem. 266: 17445-17453 (1991) showed that TIMP-2 will bind to pAPMA-activated rabbit fibroblast interstitial collagenase in complexes that are stable in SDS. The 52 kDa inactive precursor was cleaved to an active 42 kDa protein by the organomercurial. Although the active protein primarily degrades type I, II and III collagen, it will also degrade gelatin to a lesser degree.

[0160] Conditioned medium (CM) from rabbit synovial fibroblasts contains interstitial collagenase as well as 72 kDa

type IV gelatinase. The CM was incubated in 5 µl of 50 mM Tris-HCl, 200 mM NaCl, 10 mM CaCl₂, pH 7.5 for 15 minutes in either the presence or absence of TIMP-2 (according to EP 0 398 753), TIMP-2Δ or the Figure 1 TIMP-3. Note that TIMP-2Δ refers to a truncated biologically active form of TIMP-2 with amino acids 128-194 of the mature protein deleted. Tolley et al., J. Mol. Biol. 229: 1163-1164 (1993); Willenbrock et al., Biochemistry 32: 4430-4437 (1993). It has previously been shown that TIMP-2 interacts preferentially with 72 kDa procollagenase but that these complexes were not stable in 0.1% (w/v) SDS. Stetler-Stevenson, J. Biol. Chem., 264: 17374-17378 (1989). The TIMP-3 tested was the dialyzed TIMP-3 of Figure 3.

[0161] In the absence of TIMPs, 2 zones of clearing are visible when CM from rabbit synovial fibroblasts is run on a 10% acrylamide, 0.1% gelatin gel. Figure 15. One of the bands corresponds to 42 kDa pAPMA-activated interstitial collagenase. This clearing was absent in the presence of CM incubated with TIMP-2, TIMP-2Δ, or the Figure 1 TIMP-3. The other zone of clearing was not affected, meaning that it did not form as SDS-stable complex with the TIMP. In a separate experiment using the present methods (data not shown) a zone of clearing generated by the collagenase in medium conditioned by COS-7 cells was not inhibited by the presence of TIMP-2, TIMP-2Δ or TIMP-3.

15 EXAMPLE 4

Preparation of TIMP-3 Polypeptide Analogs and Nucleic Acid Variants

[0162] The amino acid sequence of full length TIMP-3 is presented in Figure 1. Using the numbering of Figure 1, the full length sequence is 188 amino acids long. The amino acid sequence at -23 through -1 is a leader sequence, and thus the pro version of the polypeptide is 211 amino acids in length.

[0163] The coding region of the TIMP-3 DNA of Figure 1 is -69 through position 564 of the nucleic acid sequence illustrated.

[0164] Alternatively, for either variant, one may construct a signal peptide sequence for eucaryotic cell expression. As can be seen from Figure 16, two additional cDNA clones have been isolated, TIMP3clone2, Seq. ID Nos. 14, 15 (ATCC Accession No. 69456) and TIMP3HCM-3 Seq. ID Nos. 16, 17 (ATCC Accession No. 69453). These clones represent natural variants. Timp3clone2 lacks part of the region encoding the N-terminus of the leader sequence of TIMP3clone7. As such, this would be preferably expressed in a prokaryote, such as E. coli. TIMP3HCM-3 lacks a portion of the region encoding the NH₂-terminus of the mature protein. Since this clone lacks the hydrophobic leader sequence, it would be preferably expressed in a prokaryote, such as E. coli.

[0165] Figure 16 shows that there are some differences among the three cDNA clones. At nucleotide 320, there is an A in TIMP3clone 2 and a T in TIMP3clone 7. This would result in a change in the amino acid sequence from a *trp* to an *arg* at position 14 in the hydrophobic leader sequence. This difference may be a cloning artifact due to its location at the 5' end of that clone. ChIMP-3 also has a *trp* at this position. Another divergence can be found at base 529, in which clone 2 has a C and clones 7 and HCM-3 have a T. This polymorphism does not result in an amino acid change because both CAT and CAC encode *his*. Other polymorphisms are found in or near the poly A tail. The poly A tail of HCM-3 is preceded by a single G, whereas in the other 2 clones it is preceded by GG. The poly A tail of clone 7 is 15 bases long and the poly A tail of HCM-3 is 18 bases long. The poly A tail of clone 2 is 17 bases long, is interrupted by 3 other bases, and is followed by 32 nucleotides of additional 5' untranslated sequence.

[0166] PCR product 29 (TIMP3PCR29 Seq. ID Nos. 18, 19, see Figure 16) was also obtained from the human fetal kidney cDNA screening, using one insert specific primer and one vector specific primer as follows: Seq. ID No. 21 (496-16) (CLWTDM forward):

45 Seq. ID No. 21 (496-16) (CLWTDM forward) :

5'- CGG AAT TCT GTC TCT GGA CCG ACA TGC TCT CC 3'

50 Seq. ID No. 20 (489-23) (lambda gt11 reverse) :

5' GAC ACC AGA CCA ACT GGT AAT G 3'

[0167] As can be seen from Figure 16, this may represent a naturally occurring C-terminal variant. At Figures 16B, bottom, to 16C, top, differences in amino acid sequence between TIMP3clone7 and TIMP3PCR29 are indicated. TIMP3PCR29, cloned into pUC19 and placed into E. coli has been deposited at the ATCC with accession no. 69532. A full cDNA clone encompassing this PCR product has not been found in the fetal kidney cDNA library, however. It is unknown if TIMP3PCR29 represents a full or partial variant or a PCR artifact.

[0168] Other TIMP-3 analogs may be prepared. One type of analog is a truncated form which exhibits binding to the

portion of a metalloproteinase which binds zinc. As indicated *supra*, the conserved region for this zinc binding domain may be represented by H E X G H, wherein X is either F or L. By analogy to TIMP-2 deletion analogs which have been prepared, TIMP-3 analogs maintaining enzyme inhibition activity may also be prepared.

[0169] Figure 17 is an illustration of the proposed secondary structure for the TIMP family of proteins. See Alexander et al., Extracellular Matrix Degradation, in, Cell Biology of Extracellular Matrix (2d ed., Hay, ed.), Plenum Press, New York, pp. 255-302. As can be seen, the six C-terminal cysteines form a secondary structure which is somewhat separate from the structure formed by the region encompassing the first six cysteines. Previously, TIMP-2 analogs lacking the C-terminus up to and including the 6th cysteine in from the C-terminus have been shown to have activity. Willenbrock et al., Biochemistry 32: 4330-4337 (1993). TIMP-3 analogs lacking one or more of the C-terminal cysteines are those having the sequence (referring to the numbering of Figure 1) of 1-121, and any of 1-122 through 1-188. Additions, deletions, and substitutions may also be made to amino acids 122-188, as well as attachment of chemical moieties, such as polymers.

[0170] While the present invention has been described in terms of preferred embodiments, it is understood that variations and modifications will occur to those skilled in the art. Therefore, it is intended that the appended claims cover all such equivalent variations which come within the scope of the invention as claimed.

SEQUENCE LISTING

[0171]

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: Tissue Inhibitor Metalloproteinase Type Three (TIMP-3) Composition and Methods

(iii) NUMBER OF SEQUENCES: 21

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Amgen Inc./Patent Operations/KMP
 (B) STREET: 1840 Dehavilland Drive
 (C) CITY: Thousand Oaks
 (D) STATE: California
 (E) COUNTRY: USA
 (F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
 (B) COMPUTER: IBM PC compatible
 (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
 (B) FILING DATE:
 (C) CLASSIFICATION:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

5 CGGAATTCGT NATHMGNGC

19

(2) INFORMATION FOR SEQ ID NO:2:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: DNA (genomic)

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25 CGGGATCCCA TRTCNGTCCA DATRCA

26

(2) INFORMATION FOR SEQ ID NO:3:

25 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: DNA (genomic)

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

40 CGGGATCCRT CNGTCCADAT RCA

23

(2) INFORMATION FOR SEQ ID NO:4:

45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

50 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

55 GTTTTCCCCAG TCACGACG

18

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

5
 (A) LENGTH: 18 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GAATTGTGAG CGGATAAC**18**

15 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

20
 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid.
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CCGAATTCTG GTCTACACCA TCAAGC**26**

30 (2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

35
 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CATGTCGGTC CAGAGACACT CG**22**

45 (2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

50
 (A) LENGTH: 31 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AACAAACATA TGTGCACATG CTCGCCAGC C

31

(2) INFORMATION FOR SEQ ID NO:9:

5

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 32 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

15

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

CGGGATCCTA TTAGGGGTCT GTGGCATTTGA TG

32

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

25

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ACCACTGGCG GTGATACTGA G

21

35

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

40

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

45

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

50

GGTCATTACT GGACCGGATC

20

(2) INFORMATION FOR SEQ ID NO:12:

55

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1240 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

10	GGCGGGGGGC GCTCAGACGG CTTCTCCCTC TCCTCTTGCT CCTCCAAGCT CCTGCTCCTT	60
	CGCCGGGAGC CCGCCCGCCG AGTCTGCGC CAGCGCCGAG GCAGCCTCGC TGCGCCCCAT	120
	CCCGTCCCGC CGGGCACTCG GAGGGCAGCG CGCCGGAGGC CAAGGTTGCC CCGCACGGCC	180
	CGGCGGGCGA GCGAGCTCGG GCTGCAGCAG CCCCGCCGGC GGCGCGCACG GCAACTTGG	240
15	AGAGGCGAGC AGCAGCCCCG GCAGCGCCGG CAGCAGCGGC AATGACCCCT TGGCTCGGGC	300
	TCATCGTGCT CCTGGGCAGC TGGAGCCTGG GGGACTGGGG CGCCGAGGCG TGACATGCT	360
	CGCCCAGCCA CCCCCAGGAC GCCTTCIGCA ACTCCGACAT CGTGATCCGG GCCAAGGTGG	420
20	TGGGGAAGAA GCTGGTAAAG GAGGGGCCCT TCGGCACGCT GGTCTACACC ATCAAGCAGA	480
	TGAAGATGTA CCGAGGCTTC ACCAAGATGC CCCATGTGCA GTACATCCAT ACGGAAGCTT	540
	CCGAGAGTCT CTGTGGCCCT AAGCTGGAGG TCAACAAGTA CCAGTACCTG CTGACAGGTC	600
25	GCGTCTATGA TGGCAAGATG TACACGGGGC TGTGCAACTT CGTGGAGAGG TGGGACCAGC	660
	TCACCCCTCTC CCAGCGCAAG GGGCTGAAC ATCGGTATCA CCTGGGTGT AACFGCAAGA	720
	TCAAGTCCTG CTACTACCTG CCTTGCTTTG TGACTTCAA GAACGAGTGT CTCTGGACCG	780
30		
	ACATGCTCTC CAATTCGGT TACCCCTGGCT ACCAGTCCAA ACACATACGCC TGCAATCCGGC	840
	AGAAGGGCGG CTACTGCAGC TGGTACCGAG GATGGGCCCC CCCGGATAAA AGCATCATCA	900
35	ATGCCACAGA CCCCTGAGCG CCAGACCCCTG CCCCACCTCA CTTCCCTCCC TTCCCGCTGA	960
	GCTTCCCTTG GACACTAACT CTTCCAGAT GATGACAATG AAATTAGTGC CTGTTTTCTT	1020
	GCAAATTAG CACTTGGAAC ATTTAAAGAA AGGTCTATGC TGTCAATATGG GGTTTATTGG	1080
40	GAACATACCT CCTGGCCCCA CCCTGCCCT TCTTTTTGGT TTTGACATCA TTCATTTCCA	1140
	CCTGGGAATT TCTGGTGCCA TGCCAGAAAG AATGAGGAAC CTGTATTCCCT CTTCTTCGTG	1200
	ATAATATAAT CTCTATTTT TTAGGAAAAA AAAAAAAA	1240

45 (2) INFORMATION FOR SEQ ID NO:13:

50 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 211 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Thr Pro Trp Leu Gly Leu Ile Val Leu Leu Gly Ser Trp Ser Leu
 1 5 10 15

5 Gly Asp Trp Gly Ala Glu Ala Cys Thr Cys Ser Pro Ser His Pro Gln
 20 25 30

Asp Ala Phe Cys Asn Ser Asp Ile Val Ile Arg Ala Lys Val Val Gly
 35 40 45

10 Lys Lys Leu Val Lys Glu Gly Pro Phe Gly Thr Leu Val Tyr Thr Ile
 50 55 60

Lys Gln Met Lys Met Tyr Arg Gly Phe Thr Lys Met Pro His Val Gln
 65 70 75 80

15 Tyr Ile His Thr Glu Ala Ser Glu Ser Leu Cys Gly Leu Lys Leu Glu
 85 90 95

Val Asn Lys Tyr Gln Tyr Leu Leu Thr Gly Arg Val Tyr Asp Gly Lys
 100 105 110

20 Met Tyr Thr Gly Leu Cys Asn Phe Val Glu Arg Trp Asp Gln Leu Thr
 115 120 125

25 Leu Ser Gln Arg Lys Gly Leu Asn Tyr Arg Tyr His Leu Gly Cys Asn
 130 135 140

30 Cys Lys Ile Lys Ser Cys Tyr Tyr Leu Pro Cys Phe Val Thr Ser Lys
 145 150 155 160

Asn Glu Cys Leu Trp Thr Asp Met Leu Ser Asn Phe Gly Tyr Pro Gly
 165 170 175

35 Tyr Gln Ser Lys His Tyr Ala Cys Ile Arg Gln Lys Gly Gly Tyr Cys
 180 185 190

Ser Trp Tyr Arg Gly Trp Ala Pro Pro Asp Lys Ser Ile Ile Asn Ala
 195 200 205

40 Thr Asp Pro
 210

(2) INFORMATION FOR SEQ ID NO:14:

45 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 963 base pairs
- (B) TYPE: nucleic acid
- 50 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

	CAGGAGCCTG GGGGACTGGG CGGCCGAGGC GTGCACATGC TCGCCCAGCC ACCCCCAGGA	60
5	CCCCCTCTGC AACTCCGACA TCGTGATCCG GGCCAAGGTG GTGGGGAAAGA AGCTGGTAAA	120
	GGAGGGGCCCG TTGGCACGC TGTTCTACAC CATCAAGCAG ATGAAGATGT ACCGAGGCTT	180
	CACCAAGATG CCCCATGTGC AGTACATCCA CACGGAAGCT TCCGAGAGTC TCTGTGGCCT	240
10	TAAGCTGGAG GTCAACAAGT ACCAGTACCT GCTGACAGGT CGCGTCTATG ATGGCAAGAT	300
	GTACACGGGG CTGTGCAACT TCGTGAGAG GTGGGACAG CTCACCCCTCT CCCAGCGCAA	360
	GGGGCTGAAC TATCGGTATC ACCTGGGTIG TAACTGCAAG ATCAAGTCCT GCTACTACCT	420
15	CCCTTGCTTT GTGACTTCCA AGAACGAGTG TCTCTGGACC GACATGCTCT CCAATTTCGG	480
	TTACCCCTGGC TACCAAGTCCA AACACTACGC CTGCATCCGG CAGAAGGGCG GCTACTGCAG	540
	CTGGTACCGA GGATGGGCCCG CCCCGGATAA AAGCATCATC AATGCCACAG ACCCCTGAGC	600
20	GCCAGACCCCT GCCCCCACCTC ACTTCCCTCC CTTCGGCTG AGCTTCCCTT GGACACTAAC	660
	TCTTCCCAGA TGATGACAAT GAAATTAGTG CCTGTTTCT TGCAAATTAA GCACCTGGAA	720

25	CATTTAAAGA AAGGTCTATG CTGTCATATG GGGTTTATTG GGAACATATCC TCCTGGCCCC	780
	ACCCCTGCCCG TTCTTTTGG TTTTGACATC ATTCAATTCC ACCTGGGAAT TTCTGGTGGCC	840
	ATGCCAGAAA GAATGAGGAA CCTGTATTC TCTCTTCGT GATAATATAA TCTCTATTTT	900
30	TTTAGGAAAA CAAAAATGAA AAACACTACTCC ATTTGAGGAT TGTAATTCCC AACACCCACCT	960
	GCT	963

(2) INFORMATION FOR SEQ ID NO:15:

35 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 198 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

50

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Arg Ser Leu Gly Asp Trp Gly Ala Glu Ala Cys Thr Cys Ser Pro Ser
 1 5 10 15

5 His Pro Gln Asp Ala Phe Cys Asn Ser Asp Ile Val Ile Arg Ala Lys
 20 25 30

Val Val Gly Lys Lys Leu Val Lys Glu Gly Pro Phe Gly Thr Leu Val
 35 40 45

10 Tyr Thr Ile Lys Gln Met Lys Met Tyr Arg Gly Phe Thr Lys Met Pro
 50 55 60

15 His Val Gln Tyr Ile His Thr Glu Ala Ser Glu Ser Leu Cys Gly Leu
 65 70 75 80

Lys Leu Glu Val Asn Lys Tyr Gln Tyr Leu Leu Thr Gly Arg Val Tyr
 85 90 95

20 Asp Gly Lys Met Tyr Thr Gly Leu Cys Asn Phe Val Glu Arg Trp Asp
 100 105 110

Gln Leu Thr Leu Ser Gln Arg Lys Gly Leu Asn Tyr Arg Tyr His Leu
 115 120 125

25 Gly Cys Asn Cys Lys Ile Lys Ser Cys Tyr Tyr Leu Pro Cys Phe Val
 130 135 140

Thr Ser Lys Asn Glu Cys Leu Trp Thr Asp Met Leu Ser Asn Phe Gly
 145 150 155 160

30 Tyr Pro Gly Tyr Gln Ser Lys His Tyr Ala Cys Ile Arg Gln Lys Gly
 165 170 175

35 Gly Tyr Cys Ser Trp Tyr Arg Gly Trp Ala Pro Pro Asp Lys Ser Ile
 180 185 190

Ile Asn Ala Thr Asp Pro
 195

40

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:

45

- (A) LENGTH: 820 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

55

	GGGAAGAACCGGTTAAAGGA	GGGGCCCTTC	GGCACCGCTGG	TCTACACCATTAAAGCAGATG	60
5	AAGATGTACCAAGGCTTCAC	CAAGATGCCCATG	CATGTGCAGT	ACATCCATACGGAAAGCTTCC	120
	GAGAGTCTCTG	TGAGGCTTAA	GCTGGAGGTC	AACAAGTACCAAGTACCTGCTGACAGGTCGC	180
	GTCTATGATG	GCAAGATGTA	CACGGGGCTG	TGCAACTTCG	240
10	ACCCCTCTCCCAGCGCAAGGG	GCTGAACTATCGGTATCACC	TGGGTTGTAA	CTGCAAGATC	300
	AAGTCCTGCTACTACCTGCC	TTGCTTTGTG	ACTTCCAAGA	ACGAGTGCTCTGGACCGAC	360
15	■GCTCTCCAATTCGGTTACCTGGCTAC	CAGTCCAAAC	ACTACGCCCTG	CATCCGGCAG	420
	AAGGGCGGCTACTGCAGCTG	GTACCGAGGA	TGGGCCCCCCC	CGGATAAAAGCATCATCAAT	480
	GCCACAGACCCTTGAGCGCC	AGACCCCTGCC	CCACCTCACT	TCCCTCCCTTCCCCTGAGC	540
20	TTCCCTTGGCACTAACTCT	TCCCAGATGA	TGACAATGAA	ATTAGTGCTGTTITCTTGC	600
	AAATTTAGCACTTGGAACAT	TTAAAGAAAG	GTCTATGCTG	TCATATGGGG	660
	ACTATCCTCC	TGGCCCCACC	CTGCCCCCTTC	TTTTGGTTTGACATCATT	720
	TGGGAATTTC	TGGTGCCATG	CCAGAAAGAA	TGAGGAACCT	780
25	AATATAATCT	CTATTTTTT	AGAAAAAAA	AAAAAAA	820

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 164 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

40

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Gly Lys Lys Leu Val Lys Glu Gly Pro Phe Gly Thr Leu Val Tyr Thr
 1 5 10 15

Ile Lys Gln Met Lys Met Tyr Arg Gly Phe Thr Lys Met Pro His Val
 5 20 25 30

Gln Tyr Ile His Thr Glu Ala Ser Glu Ser Leu Cys Gly Leu Lys Leu
 35 40 45

Glu Val Asn Lys Tyr Gln Tyr Leu Leu Thr Gly Arg Val Tyr Asp Gly
 10 50 55 60

Lys Met Tyr Thr Gly Leu Cys Asn Phe Val Glu Arg Trp Asp Gln Leu
 15 65 70 75 80

Thr Leu Ser Gln Arg Lys Gly Leu Asn Tyr Arg Tyr His Leu Gly Cys
 85 90 95

Asn Cys Lys Ile Lys Ser Cys Tyr Tyr Leu Pro Cys Phe Val Thr Ser
 20 100 105 110

Lys Asn Glu Cys Leu Trp Thr Asp Met Leu Ser Asn Phe Gly Tyr Pro
 115 120 125

Gly Tyr Gln Ser Lys His Tyr Ala Cys Ile Arg Gln Lys Gly Gly Tyr
 25 130 135 140

Cys Ser Trp Tyr Arg Gly Trp Ala Pro Pro Asp Lys Ser Ile Ile Asn
 145 150 155 160

Ala Thr Asp Pro
 30

(2) INFORMATION FOR SEQ ID NO:18:

35 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 92 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: DNA (genomic)

45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CTCTGGACCG ACATGCTCTC CAATTCGGT TACCCTGGCT ACCAGTCCAA ACACTACACA	60
TGCTCGCCCA GCCACCCCCG CACCGCGCTCC CG	92

50 (2) INFORMATION FOR SEQ ID NO:19:

55 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

5 Leu Trp Thr Asp Met. Leu Ser Asn Phe Gly Tyr Pro Gly Tyr Gln Ser
1 5 10 15

Lys His Tyr Thr Cys Ser Pro Ser His Pro Arg Thr Arg Ser Thr
 20 25 30

10

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 22 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

25 GACACCAGAC CAACTGGTAA TG

22

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

40

32

Claims

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1. An isolated DNA sequence comprising a sequence that encodes a protein comprising the amino acid sequence from amino acid +1 to +188 shown in Figure 1.
2. The DNA sequence of claim 1 wherein the DNA sequence is a cDNA sequence.
3. The DNA sequence of claim 1 wherein the DNA sequence is a genomic DNA sequence.
4. The DNA sequence of claim 1 which encodes the amino acid sequence shown in Figure 1.
5. The DNA sequence of claim 1, the expression of which is optimized by the inclusion of one or more codons preferred for expression in bacterial cells.
6. The DNA sequence of claim 1 the expression of which is optimized by the inclusion of one or more codons preferred

for expression in mammalian cells.

7. The DNA sequence of claim 1 the expression of which is optimized by the inclusion of one or more codons preferred for expression in yeast cells.

5

8. The DNA sequence of claim 1 covalently associated with a detectable label substance.

9. A antisense DNA with respect to the DNA sequence of claim 1.

10. A DNA sequence as set forth in Figure 1 or Figure 16, said sequence encoding at least amino acids 1-188 as set forth in Figure 1, and optionally encoding an additional methionyl residue at the -1 position.

11. The DNA sequence of claim 10 covalently associated with a detectable label substance.

15. 12. An antisense DNA with respect to the DNA sequence of claim 10.

13. A DNA sequence of claim 10, the expression of which is optimized by the inclusion of one or more codons preferred for expression in bacterial cells.

20. 14. The DNA sequence of claim 10 the expression of which is optimized by the inclusion of one or more codons preferred for expression in mammalian cells.

15. The DNA sequence of claim 10 the expression of which is optimized by the inclusion of one or more codons preferred for expression in yeast cells.

25

16. A vector containing the DNA sequence of claim 1.

17. The vector of claim 16 wherein said vector is a plasmid vector.

30. 18. The vector of claim 16 wherein said vector is a viral vector.

19. The vector of claim 18 wherein said viral vector is selected from the group consisting of a bacteriophage vector, a retroviral vector, and an adenoviral vector.

35. 20. The vector containing the DNA sequence of claim 10.

21. The vector of claim 20 wherein said vector is a plasmid vector.

22. The vector of claim 20 wherein said vector is a viral vector.

40

23. The vector of claim 22 wherein said viral vector is selected from the group consisting of a bacteriophage vector, a retroviral vector, and an adenoviral vector.

45. 24. A procaryotic or eucaryotic isolated host cell transformed or transfected with the DNA sequence of claim 1 in a manner allowing the host cell to express said polypeptide product.

25. A procaryotic or eucaryotic isolated host cell transformed or transfected with the DNA sequence of claim 10 in a manner allowing the host cell to express said polypeptide product.

50. 26. A polypeptide product of expression of the DNA sequence of claim 1 in a procaryotic or eucaryotic host cell.

27. The polypeptide of claim 26 wherein said polypeptide binds the amino acid sequence H E X G H where X is either F or L.

55. 28. A polypeptide product of expression of the DNA sequence of claim 10 in a procaryotic or eucaryotic host cell.

29. The host cell selected from the group consisting of those with American Type Culture Collection™ designations (Timp3clone7/pCFM, Timp3clone7/puC19, Timp3clone2/puC19, Timp3HCM3, TimP3PCR29) 69455, 69454,

69456, 69453, and 69532.

30. A process for the production of a polypeptide having the amino acid sequence shown in Figure 1 comprising: growing, under suitable conditions, prokaryotic or eukaryotic isolated host cells transformed or transfected with the DNA sequence of claim 1 or 10, and isolating desired polypeptide products of the expression of said DNA sequences.
5
31. A composition containing the DNA sequence of claim 1 and a pharmaceutically acceptable carrier.
10
32. A composition containing the DNA sequence of claim 10 and a pharmaceutically acceptable carrier.
15
33. The composition of claim 31 wherein said carrier is selected from the group consisting of a lipid solution carrier, a liposome, and a polypeptide.
15
34. The composition of claim 32 wherein said carrier is selected from the group consisting of a lipid solution carrier, a liposome, and a polypeptide.
20
35. Purified and isolated polypeptide having the amino acid sequence shown in Figure 1.
25
36. A purified and isolated polypeptide comprising the amino acid sequence from amino acid +1 to +188 shown in Figure 1 immunological properties of a polypeptide having the amino acid sequence shown in Figure 1, wherein the biological property is the inhibition of a metalloproteinase or the binding to the extracellular matrix material and wherein the immunological property is the raising of antibodies specifically binding to a polypeptide having the amino acid sequence shown in Figure 1.
30
37. The polypeptide of claim 36 wherein said polypeptide is the product of prokaryotic or eukaryotic expression of an exogenous DNA sequence.
35
38. The polypeptide of claim 37 wherein said exogenous DNA sequence is a cDNA sequence.
30
39. The polypeptide of claim 37 wherein said exogenous DNA sequence is a genomic DNA sequence.
40
40. The polypeptide of claim 37 wherein said polypeptide has the amino acid sequence shown in Figure 1.
45
41. The polypeptide of claim 37 wherein said exogenous DNA sequence is carried on an autonomously replicating DNA plasmid or viral vector.
40
42. A purified and isolated polypeptide having the amino acid sequence of amino acids 1-188 as presented in Figure 1, optionally having a methionyl residue at position -1.
45
43. The polypeptide of claim 35 or 36 further characterized by being covalently associated with a detectable label substance.
45
44. A polypeptide as set forth in Figure 1, optionally having a methionyl residue at position -1, lacking one or more of the six C-terminal cysteine residues.
50
45. A polypeptide as set forth in Figure 1, optionally having a methionyl residue at position -1; having the amino acid sequence 1-121 and optionally all or part of amino acids 122-188.
50
46. The polypeptide of claim 45 having the capacity to bind the zinc binding domain of collagenase.
55
47. The polypeptide of claim 45 having a chemical modification located at one or more of amino acids 122-188.
55
48. A pharmaceutical composition comprising a purified and isolated polypeptide having the amino acid sequence shown in Figure 1 in a pharmaceutically acceptable diluent, adjuvant or carrier.
55
49. An article of manufacture comprising a packaging material and a pharmaceutical agent, wherein said pharmaceutical agent contains a polypeptide having the amino acid sequence shown in Figure 1 and Wherein said packaging

material comprises a label which indicates that said pharmaceutical agent may be used for an indication selected from the group consisting of cancer, inflammation, arthritis, dystrophic epidermolysis bullosa, periodontal disease, ulceration, emphysema, bone disorders, scleroderma, wound healing, erythrocyte deficiencies, cosmetic tissue reconstruction, fertilization or embryo implant modulation and nerve cell disorders.

- 5 50. An article of manufacture comprising a packaging material and a pharmaceutical agent, wherein said pharmaceutical agent contains a DNA encoding the amino acid sequence shown in Figure 1 and wherein said packaging material comprises a label which indicates that said pharmaceutical composition may be used for an indication benefitting from genetic therapy using such DNA.
- 10 51. The article of manufacture of claim 50 wherein said indication is emphysema.
- 15 52. A kit containing a preparation of a polypeptide having the amino acid sequence shown in Figure 1 and one or more additional compositions beneficial for the treatment of a disorder involving the degradation of extracellular matrix.
- 20 53. The kit of claim 52 wherein said additional composition is selected from the group consisting of: metalloproteinases, serine proteases, inhibitors of matrix degrading enzymes, intracellular enzymes, cell adhesion modulators, and factors regulating the expression of extracellular matrix degrading proteinases and their inhibitors.
- 25 54. The kit of claim 52 wherein said additional composition is selected from the group consisting of E-selectins, integrins, L-selectins, chemokines, and chemoattractants.
- 30 55. The kit of claim 52 wherein said additional composition is selected from the group consisting of BDNF, NT-3, NGF, CNTF, and NDF.
- 35 56. The kit of claim 53 wherein said additional composition is selected from the group consisting of collagenases, PMN collagenase, stromelysin I, II/transin, matrilysin, invadolysin, PUMP-1, UPA, TPA, and plasmin.
- 40 57. The kit of claim 53 wherein said additional composition is selected from the group consisting of α_2 -macroglobulin, pregnancy zone protein, ovostatin, α_1 -proteinase inhibitor, α_2 -antiplasmin, aprotinin, protease nexin-1, PAI-1, PAI-2, TIMP-1 and TIMP-2.
- 45 58. The kit of claim 53 wherein said additional composition is selected from the group consisting of lysosomal enzymes, glycosidases and cathepsins.
- 50 59. The kit of claim 53 wherein said additional composition is a cell adhesion modulator.
- 55 60. The kit of claim 53 wherein said additional composition is a factor regulating expression of extracellular matrix degrading proteinases and their inhibitors.
- 60 61. The kit of claim 53 wherein said additional composition is selected from the group consisting of an interleukin, TNF α , TGF- β , glucocorticoids, retinoids, EPO, SCF, M-CSF, IGF-I, IGF-II, EGF, an FGF, KGF, PDGF, an interferon, protein kinase C, and inositol phosphatases.
- 65 62. A kit including a DNA encoding the amino acid sequence shown in Figure 1 and one or more additional factors affecting the ex vivo growth of cells transformed or transfected with said DNA
- 70 63. The kit of claim 62 including SCF.
- 75 64. A monoclonal antibody directed against a polypeptide having the amino acid sequence shown in Figure 1.
- 80 65. A kit containing a monoclonal antibody directed against a polypeptide having the amino acid sequence shown in Figure 1.

55 Patentansprüche

- 1. Eine isolierte DNA-Sequenz, umfassend eine Sequenz, die für ein Protein kodiert, umfassend die in Figur 1 ge-

zeigte Aminosäuresequenz von Aminosäure +1 bis +188.

2. Die DNA-Sequenz nach Anspruch 1, wobei die DNA-Sequenz eine cDNA-Sequenz ist.
- 5 3. Die DNA-Sequenz nach Anspruch 1, wobei die DNA-Sequenz eine genomische DNA-Sequenz ist
4. Die DNA-Sequenz nach Anspruch 1, die für die in Figur 1 gezeigte Aminosäuresequenz kodiert.
- 10 5. Die DNA-Sequenz nach Anspruch 1, deren Expression durch die Einbeziehung von ein oder mehreren Kodons optimiert ist, die für die Expression in bakteriellen Zellen bevorzugt sind.
6. Die DNA-Sequenz nach Anspruch 1, deren Expression durch die Einbeziehung von ein oder mehreren Kodons optimiert ist, die für die Expression in Säugetierzellen bevorzugt sind.
- 15 7. Die DNA-Sequenz nach Anspruch 1, deren Expression durch die Einbeziehung von ein oder mehreren Kodons optimiert ist, die für die Expression in Hefezellen bevorzugt sind.
8. Die DNA-Sequenz nach Anspruch 1, die kovalent mit einer nachweisbaren Markierungssubstanz assoziiert ist.
- 20 9. Eine Antisense-DNA bezüglich der DNA-Sequenz nach Anspruch 1.
10. Eine DNA-Sequenz, wie in Figur 1 oder Figur 16 gezeigt, wobei diese Sequenz mindestens für die Aminosäuren 1-188, wie in Figur 1 gezeigt, kodiert und gegebenenfalls für einen zusätzlichen Methionylrest an der -1-Position kodiert.
- 25 11. Die DNA-Sequenz nach Anspruch 10, die kovalent mit einer nachweisbaren Markierungssubstanz assoziiert ist.
12. Eine Antisense-DNA bezüglich der DNA-Sequenz nach Anspruch 10.
- 30 13. Eine DNA-Sequenz nach Anspruch 10, deren Expression durch die Einbeziehung von ein oder mehreren Kodons optimiert ist, die für die Expression in Bakterienzellen bevorzugt sind.
14. Die DNA-Sequenz nach Anspruch 10, deren Expression durch die Einbeziehung von ein oder mehreren kodons optimiert ist, die für die Expression in Säugetierzellen bevorzugt sind.
- 35 15. Die DNA-Sequenz nach Anspruch 10, deren Expression durch die Einbeziehung von ein oder mehreren Kodons optimiert ist, die für die Expression in Hefezellen bevorzugt sind.
16. Ein Vektor, enthaltend die DNA-Sequenz nach Anspruch 1.
- 40 17. Der Vektor nach Anspruch 16, wobei der Vektor ein Plasmidvektor ist.
18. Der Vektor nach Anspruch 16, wobei der Vektor ein viraler Vektor ist.
- 45 19. Der Vektor nach Anspruch 18, wobei der virale Vektor ausgewählt ist aus der Gruppe bestehend aus einem Bakteriophagen-Vektor, einem retroviroalen Vektor und einem adenoviralen Vektor.
20. Der Vektor, enthaltend die DNA-Sequenz nach Anspruch 10.
- 50 21. Der Vektor nach Anspruch 20, wobei der Vektor ein Plasmidvektor ist.
22. Der Vektor nach Anspruch 20, wobei der Vektor ein viraler Vektor ist.
- 55 23. Der Vektor nach Anspruch 22, wobei der virale Vektor ausgewählt ist aus der Gruppe bestehend aus Bakteriophage-Vektor, einem retroviroalen Vektor und einem adenoviralen Vektor.
24. Eine prokaryotische oder eukaryotische, isolierte Wirtszelle, die mit der DNA-Sequenz nach Anspruch 1 derart transformiert oder transfiziert ist, dass die Wirtszelle das Polypeptidprodukt exprimieren kann.

25. Eine prokaryotische oder eukaryotische, isolierte Wirtszelle, die mit der DNA-Sequenz nach Anspruch 10 derart transformiert oder transfiziert ist, dass die Wirtszelle das Polypeptidprodukt exprimieren kann.

5 26. Ein Polypeptid-Expressionsprodukt der DNA-Sequenz nach Anspruch 1 in einer prokaryotischen oder eukaryotischen Wirtszelle.

27. Das Polypeptid nach Anspruch 26, wobei das Polypeptid die Aminosäuresequenz HEXGH bindet, wobei X entweder F oder L ist.

10 28. Ein Polypeptid-Expressionsprodukt der DNA-Sequenz nach Anspruch 10 in einer prokaryotischen oder eukaryotischen Wirtszelle.

29. Die Wirtszelle, ausgewählt aus der Gruppe bestehend aus denjenigen mit den American Type Culture Collection™-Bezeichnungen (Timp3clone7/pCFM, Timp3clone7/puC19, Timp3clone2/puC19, Timp3HCM3, TimpP3PCR29) 69455, 69454, 69456, 69453 und 69532.

15 30. Ein Verfahren für die Herstellung eines Polypeptids, das die in Figur 1 gezeigte Aminosäuresequenz besitzt, umfassend: Wachsen von prokaryotischen oder eukaryotischen, isolierten Wirtszellen, die mit der DNA-Sequenz nach Anspruch 1 oder 10 transformiert oder transfiziert sind, unter geeigneten Bedingungen und Isolierung der gewünschten Polypeptid-Expressionsprodukte der DNA-Sequenzen.

20 31. Eine Zusammensetzung, enthaltend die DNA-Sequenz nach Anspruch 1 und einen pharmazeutisch verträglichen Träger.

25 32. Eine Zusammensetzung, enthaltend die DNA-Sequenz nach Anspruch 10 und einen pharmazeutisch verträglichen Träger.

33. Die Zusammensetzung nach Anspruch 31, wobei der Träger ausgewählt ist aus der Gruppe bestehend aus einem Lipidlösungs-Träger, einem Liposom und einem Polypeptid.

30 34. Die Zusammensetzung nach Anspruch 32, wobei der Träger ausgewählt ist aus der Gruppe bestehend aus einem Lipidlösungs-Träger, einem Liposom und einem Polypeptid.

35 35. Gereinigtes und isoliertes Polypeptid, das die in Figur 1 gezeigte Aminosäuresequenz besitzt.

36. Ein gereinigtes und isoliertes Polypeptid, umfassend die in Figur 1 gezeigte Aminosäuresequenz von Aminosäure +1 bis +188 und das die biologischen oder immunologischen Eigenschaften eines Polypeptids hat, das die in Figur 1 gezeigte Aminosäuresequenz besitzt, wobei die biologische Eigenschaft die Inhibierung einer Metalloproteinase oder die Bindung an das extrazelluläre Matrix-Material ist und die immunologische Eigenschaft die Generierung von Antikörpern ist, die spezifisch an ein Polypeptid binden, das die in Figur 1 gezeigte Aminosäuresequenz besitzt.

40 37. Das Polypeptid nach Anspruch 36, wobei das Polypeptid das Produkt der prokaryotischen oder eukaryotischen Expression einer exogenen DNA-Sequenz ist.

38. Das Polypeptid nach Anspruch 37, wobei die exogene DNA-Sequenz eine cDNA-Sequenz ist.

39. Das Polypeptid nach Anspruch 37, wobei die exogene DNA-Sequenz eine genomische DNA-Sequenz ist.

40. Das Polypeptid nach Anspruch 37, wobei das Polypeptid die in Figur 1 gezeigte Aminosäuresequenz besitzt.

50 41. Das Polypeptid nach Anspruch 37, wobei die exogene DNA-Sequenz sich auf einem sich autonom replizierenden DNA-Plasmid oder einem viralen Vektor befindet.

42. Ein gereinigtes und isoliertes Polypeptid, das die Aminosäuresequenz von Aminosäuren 1-188, wie in Figur 1 gezeigt, besitzt und das gegebenenfalls einen Methionylrest an Position -1 besitzt.

55 43. Das Polypeptid nach Anspruch 35 oder 36, weiterhin dadurch charakterisiert, dass es kovalent mit einer nachweisbaren Markierungssubstanz assoziiert ist.

44. Ein wie in Figur 1 gezeigtes Polypeptid, das gegebenenfalls ein Methionylrest an Position -1 besitzt und dem ein oder mehrere der sechs C-terminalen Cysteinreste fehlen.

5 45. Ein wie in Figur 1 gezeigtes Polypeptid, das gegebenenfalls ein Methionylrest an Position -1 besitzt und das die Aminosäuresequenz 1-121 und gegebenenfalls alle oder Teil der Aminosäuren 122-188 besitzt.

10 46. Das Polypeptid nach Anspruch 45, das die Kapazität besitzt, die Zink-Bindungsdomäne von Kollagenase zu binden.

15 47. Das Polypeptid nach Anspruch 45, das eine chemische Modifikation besitzt, die an einer oder mehreren der Aminosäuren 122-188 lokalisiert ist.

48. Eine pharmazeutische Zusammensetzung, umfassend ein gereinigtes und isoliertes Polypeptid, das die in Figur 1 gezeigte Aminosäuresequenz besitzt, in einem pharmazeutisch verträglichen Verdünnungsmittel, Adjuvans oder Träger.

20 49. Ein Herstellungserzeugnis, umfassend ein Verpackungsmaterial und ein pharmazeutisches Agens, wobei das pharmazeutische Agens ein Polypeptid enthält, das die in Figur 1 gezeigte Aminosäuresequenz besitzt und das Verpackungsmaterial ein Etikett umfasst, das darauf hinweist, dass das pharmazeutische Agens für eine Indikation verwendet werden kann, die ausgewählt ist aus der Gruppe bestehend aus Krebs, Entzündung, Arthritis, dystrophische Epidermolysis bullosa, parodontaler Erkrankung, Ulzeration, Emphysem, Knochenerkrankungen, Skleroderm, Wundheilung, Erythrozyt-Mängel, kosmetische Gewebswiederherstellung, Befruchtung oder Embryoimplantat-Modulation und Nervenzellerkrankungen.

25 50. Ein Herstellungserzeugnis, umfassend ein Verpackungsmaterial und ein pharmazeutisches Agens, wobei das pharmazeutische Agens eine DNA enthält, die für die in Figur 1 gezeigte Aminosäuresequenz kodiert und das Verpackungsmaterial ein Etikett umfasst, das darauf hinweist, dass die pharmazeutische Zusammensetzung für eine Indikation verwendet werden kann, die einen Nutzen ziehen kann aus einer Gentherapie unter Verwendung solcher DNA.

30 51. Das Herstellungserzeugnis nach Anspruch 50, wobei die Indikation Emphysem ist.

52. Ein Kit, enthaltend eine Zubereitung eines Polypeptids, die die in Figur 1 gezeigte Aminosäuresequenz besitzt, und eine oder mehrere Zusammensetzungen, die nützlich sind für die Behandlung einer Krankheit, die den Abbau der extrazellulären Matrix umfasst.

35 53. Der Kit nach Anspruch 52, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus: Metalloproteinasen, Serinproteasen, Inhibitoren von Matrix abbauenden Enzymen, intrazellulären Enzymen, Zelladhäsionsmodulatoren und Faktoren, die die Expression von extrazellulären Matrix abbauenden Proteininasen und ihren Inhibitoren regulieren.

40 54. Der Kit nach Anspruch 52, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus E-Selectinen, Integrinen, L-Selectinen, Chemokinen und chemischen Lockstoffen.

45 55. Der Kit nach Anspruch 52, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus BDNF, NT-3, NGF, CNTF und NDF.

56. Der Kit nach Anspruch 53, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus Kollagenasen, PMN-Kollagenase, Stromelysin I, II/Transin, Matrilysin, Invadolysin, PUMP-1, UPA, TPA und Plasmin.

50 57. Der Kit nach Anspruch 53, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus α_2 -Makroglobulin, Schwangerschaftszonenprotein (pregnancy zone protein), Ovostatin, α_1 -Proteinaseinhibitor, α_2 -Antiplasmin, Aprotinin, Protease Nexin-1, PAI-1, PAI-2, TIMP-1 und TIMP-2

55 58. Der Kit nach Anspruch 53, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus lysosomalen Enzymen, Glycosidasen und Cathepsinen.

59. Der Kit nach Anspruch 53, wobei die zusätzliche Zusammensetzung ein Zelladhäsionsmodulator ist.

60. Der Kit nach Anspruch 53, wobei die zusätzliche Zusammensetzung ein Faktor ist, der die Expression von extrazellulären Matrix abbauenden Proteinasen und deren Inhibitoren reguliert.

5 61. Der Kit nach Anspruch 53, wobei die zusätzliche Zusammensetzung ausgewählt ist aus der Gruppe bestehend aus einem Interleukin, TNF α , TGF- β , Glucocorticoide, Retinoide, EPO, SCF, M-CSF, IGF-I, IGF-II, EGF, einem FGF, KGF, PDGF, einem Interferon, Proteinkinase C und Inositolphosphatasen.

10 62. Ein Kit, einschließlich eine DNA, kodierend für die in Figur 1 gezeigte Aminosäuresequenz und einen oder mehrere zusätzliche Faktoren, die das ex vivo-Wachstum von Zellen beeinflussen, die mit der DNA transformiert oder transfiziert sind.

15 63. Der Kit nach Anspruch 62, einschließlich SCF.

16 64. Ein monoklonaler Antikörper, gerichtet gegen ein Polypeptid, das die in Figur 1 gezeigte Aminosäuresequenz besitzt

20 65. Ein Kit, enthaltend einen monoklonalen Antikörper, gerichtet gegen ein Polypeptid, das die in Figur 1 gezeigte Aminosäuresequenz besitzt.

Revendications

25 1. Séquence d'ADN isolée comprenant une séquence qui code pour une protéine comprenant la séquence d'acides aminés allant des acides aminés +1 à +188 représentée dans la figure 1.

2. Séquence d'ADN selon la revendication 1, dans laquelle la séquence d'ADN est une séquence d'ADNc.

30 3. Séquence d'ADN selon la revendication 1, dans laquelle la séquence d'ADN est une séquence d'ADN génomique.

4. Séquence d'ADN selon la revendication 1, qui code pour la séquence d'acides aminés représentée dans la figure 1.

35 5. Séquence d'ADN selon la revendication 1, dont l'expression est optimisée par l'inclusion d'un ou plusieurs codons préférés pour l'expression dans des cellules bactériennes.

6. Séquence d'ADN selon la revendication 1, dont l'expression est optimisée par l'inclusion d'un ou plusieurs codons préférés pour l'expression dans des cellules mammaliennes.

40 7. Séquence d'ADN selon la revendication 1, dont l'expression est optimisée par l'inclusion d'un ou plusieurs codons préférés pour l'expression dans des cellules de levure.

8. Séquence d'ADN selon la revendication 1, associée par covalence à une substance de marquage détectable.

45 9. ADN antisens par rapport à la séquence d'ADN selon la revendication 1.

10. Séquence d'ADN telle qu'indiquée dans la figure 1 ou la figure 16, ladite séquence codant pour au moins les acides aminés 1-188 tels qu'indiqués dans la figure 1, et en option codant pour un résidu méthionyle additionnel sur la position -1.

50 11. Séquence d'ADN selon la revendication 10, associée par covalence à une substance de marquage détectable.

12. ADN antisens par rapport à la séquence d'ADN selon la revendication 10.

55 13. Séquence d'ADN selon la revendication 10, dont l'expression est optimisée par l'inclusion d'un ou plusieurs codons préférés pour l'expression dans des cellules bactériennes.

14. Séquence d'ADN selon la revendication 10, dont l'expression est optimisée par l'inclusion d'un ou plusieurs codons

préférés pour l'expression dans des cellules mammaliennes.

15. Séquence d'ADN selon la revendication 10, dont l'expression est optimisée par l'inclusion d'un ou plusieurs codons préférés pour l'expression dans des cellules de levure.

5 16. Vecteur contenant la séquence d'ADN selon la revendication 1.

17. Vecteur selon la revendication 16, dans lequel ledit vecteur est un vecteur plasmidique.

10 18. Vecteur selon la revendication 16, dans lequel ledit vecteur est un vecteur viral.

19. Vecteur selon la revendication 18, dans lequel ledit vecteur viral est choisi dans le groupe consistant en un vecteur bactériophage, un vecteur rétroviral, et un vecteur adénoviral.

15 20. Vecteur contenant la séquence d'ADN selon la revendication 10.

21. Vecteur selon la revendication 20, dans lequel ledit vecteur est un vecteur plasmidique.

22. Vecteur selon la revendication 20, dans lequel ledit vecteur est un vecteur viral.

20 23. Vecteur selon la revendication 22, dans lequel ledit vecteur viral est choisi dans le groupe consistant en un vecteur bactériophage, un vecteur rétroviral, et un vecteur adénoviral.

25 24. Cellule hôte isolée procaryote ou eucaryote transformée ou transfectée avec la séquence d'ADN selon la revendication 1 d'une manière permettant à la cellule hôte d'exprimer ledit produit polypeptidique.

25 25. Cellule hôte isolée procaryote ou eucaryote transformée ou transfectée avec la séquence d'ADN selon la revendication 10 d'une manière permettant à la cellule hôte d'exprimer ledit produit polypeptidique.

30 26. Produit polypeptidique d'expression de la séquence d'ADN selon la revendication 1 dans une cellule hôte procaryote ou eucaryote.

27. Polypeptide selon la revendication 26, dans lequel ledit polypeptide fixe la séquence d'acides aminés H E X G H où X est F ou L.

35 28. Produit polypeptidique d'expression de la séquence d'ADN selon la revendication 10 dans une cellule hôte procaryote ou eucaryote.

40 29. Cellule hôte choisie dans le groupe consistant en celles ayant les désignations de l'American Type Culture Collection™ (Timp3clone7/pCFM, Timp3clone7/puC19, Timp3clone2/puC19, Timp3HCM3, TimP3PCR29) 69455, 69454, 69456, 69453 et 69532.

45 30. Procédé pour la production d'un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1, comprenant: la culture, dans des conditions appropriées, de cellules hôtes isolées procaryotes ou eucaryotes transformées ou transfectées avec la séquence d'ADN selon la revendication 1 ou 10, et l'isolement des produits polypeptidiques désirés de l'expression des dites séquences d'ADN.

31. Composition contenant la séquence d'ADN selon la revendication 1 et un support pharmaceutiquement acceptable.

50 32. Composition contenant la séquence d'ADN selon la revendication 10 et un support pharmaceutiquement acceptable.

33. Composition selon la revendication 31, dans laquelle ledit support est choisi dans le groupe consistant en un support en solution lipidique, un liposome et un polypeptide.

55 34. Composition selon la revendication 32, dans laquelle ledit support est choisi dans le groupe consistant en un support en solution lipidique, un liposome et un polypeptide.

35. Polypeptide purifié et isolé ayant la séquence d'acides aminés représentée dans la figure 1.

36. Polypeptide purifié et isolé comprenant la séquence d'acides aminés allant des acides aminés +1 à +188 représentée dans la figure 1 et ayant les propriétés biologiques ou immunologiques d'un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1, tandis que la propriété biologique est l'inhibition d'une métalloprotéinase ou la liaison à la matière de matrice extracellulaire et tandis que la propriété immunologique est la production d'anticorps se liant spécialement à un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1.

37. Polypeptide selon la revendication 36, dans lequel ledit polypeptide est le produit d'expression procaryote ou eucaryote d'une séquence d'ADN exogène.

38. Polypeptide selon la revendication 37, dans lequel ladite séquence d'ADN exogène est une séquence d'ADNc.

39. Polypeptide selon la revendication 37, dans lequel ladite séquence d'ADN exogène est une séquence d'ADN génomique.

40. Polypeptide selon la revendication 37, dans lequel ledit polypeptide a la séquence d'acides aminés représentée dans la figure 1.

41. Polypeptide selon la revendication 37, dans lequel ladite séquence d'ADN exogène est portée sur un vecteur plasmidique ou viral d'ADN à réPLICATION autonome.

42. Polypeptide purifié et isolé ayant la séquence d'acides aminés des acides aminés 1-188 telle que présentée dans la figure 1, ayant en option un résidu méthionyle sur la position -1.

43. Polypeptide selon la revendication 35 ou 36, caractérisé en outre comme étant associé par covalence à une substance de marquage détectable.

44. Polypeptide tel qu'indiqué dans la figure 1, ayant en option un résidu méthionyle sur la position -1, et privé d'un ou plusieurs des six résidus cystéine C-terminaux.

45. Polypeptide tel qu'indiqué dans la figure 1, ayant en option un résidu méthionyle sur la position -1, ayant la séquence d'acides aminés 1-121 et en option la totalité ou une partie des acides aminés 122-188.

46. Polypeptide selon la revendication 45, ayant la capacité à fixer le domaine de liaison de zinc de la collagénase.

47. Polypeptide selon la revendication 45, ayant une modification chimique située sur un ou plusieurs des acides aminés 122-188.

48. Composition pharmaceutique comprenant un polypeptide purifié et isolé ayant la séquence d'acides aminés représentée dans la figure 1 dans un diluant, adjuvant ou support pharmaceutiquement acceptable.

49. Article manufacturé comprenant une matière de conditionnement et un agent pharmaceutique, dans lequel ledit agent pharmaceutique contient un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1 et où ladite matière de conditionnement comprend une étiquette qui indique que ledit agent pharmaceutique peut être utilisé pour une indication choisie dans le groupe consistant en cancer, inflammation, arthrite; épidermolysie bulleuse dystrophique, affection desmodontale, ulcération, emphysème, troubles osseux, sclerodermie, cicatrisation, carence en erythrocytes, reconstruction de tissus cosmétique, fécondation ou modulation d'implant embryonnaire, et troubles des cellules nerveuses.

50. Article manufacturé comprenant une matière de conditionnement et un agent pharmaceutique, dans lequel ledit agent pharmaceutique contient un ADN codant pour la séquence d'acides aminés représentée dans la figure 1 et où ladite matière de conditionnement comprend une étiquette qui indique que ladite composition pharmaceutique peut être utilisé pour une indication tirant avantage d'une thérapie génique utilisant un tel ADN.

51. Article manufacturé selon la revendication 50, dans lequel ladite indication est l'emphysème.

52. Kit contenant une préparation d'un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1 et une ou plusieurs compositions additionnelles bénéfiques pour le traitement d'un trouble entraînant la dégradation de la matrice extracellulaire.

5 53. Kit selon la revendication 52, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en métalloprotéinases, sérine protéases, inhibiteurs d'enzymes de dégradation de matrice, enzymes intracellulaires, modulateurs d'adhésion aux cellules, et facteurs régulant l'expression des protéinases dégradant la matrice extracellulaire et de leurs inhibiteurs.

10 54. Kit selon la revendication 52, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en E-sélectines, intégrines, L-sélectines, chimiokines, et produits chimio-attractifs.

55. Kit selon la revendication 52, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en BDNF, NT-3, NGF, CNTF et NDF.

15 56. Kit selon la revendication 53, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en collagénases, PMN collagénase, stromélysine I, II/transine, matrilysine, invadolysine, PUMP-1, UPA, TPA et plasmine.

20 57. Kit selon la revendication 53, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en α_2 -macroglobuline, protéine de zone de grossesse, ovostatine, inhibiteur d' α_1 -protéinase, α_2 -antiplasmine, aprotinine, protéase nexine-1, PAI-1, PAI-2, TIMP-1 et TIMP-2.

25 58. Kit selon la revendication 53, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en enzymes lysosomiales, glycosidases et cathepsines.

59. Kit selon la revendication 53, dans lequel ladite composition additionnelle est un modulateur d'adhésion cellulaire.

30 60. Kit selon la revendication 53, dans lequel ladite composition additionnelle est un facteur régulant l'expression des protéinases dégradant la matrice extracellulaire et de leurs inhibiteurs.

61. Kit selon la revendication 53, dans lequel ladite composition additionnelle est choisie dans le groupe consistant en interieukine, TNF α , TGF- β , glucocorticoïdes, rétinoïdes, ÉPO, SCF, M-CSF, IGF-I, IGF-II, EGF, un FGF, KGF, PDGF, un interféron, la protéine kinase C, et les inositol phosphatases.

35 62. Kit incluant un ADN codant pour la séquence d'acides aminés représentée dans la figure 1 et un ou plusieurs facteurs additionnels affectant la croissance ex vivo de cellules transformées ou transfectées avec ledit ADN.

63. Kit selon la revendication 62, induant du SCF.

40 64. Anticorps monoclonal dirigé contre un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1.

65. Kit contenant un anticorps monoclonal dirigé contre un polypeptide ayant la séquence d'acides aminés représentée dans la figure 1.

45

50

55

FIG. I

GGCGGGGGCGCTCAGACGGCTTCTCCCTCCTCTTGCTCCTCCAAGCTCCTGCTCCTT	60
CGCCGGAGCCCCCCCAGTCCTGCGCCAGCGCCAGGCAGCCTCGCTGCGCCCCAT	120
CCCGTCCCAGGGCACTCGGAGGGCAGCGGCCGGAGGCCAAGGTTGCCCGCACGGCC	180
CGGCAGGGAGCGAGCTCGGCTGCAGCAGCCCCGCCGGCGCAGCAGGGCAACTTGG	240
AGAGGCAGCAGCAGCCCCGGCAGCGGCCAGCAGGGCAATGACCCCTTGGCTCGGGC	300
MetThrProTrpLeuGlyLeu	-17
	-23
TCATCGTCTCTGGGAGCTGGAGCTGGGGACTGGGGCGCCAGGGCTGCACATGCT	360
IleValLeuLeuGlySerTrpSerLeuGlyAspTrpGlyAlaGluAlaCysThrCysSer	4
	-1 +1
CGCCCAGCCACCCCCCAGGACGCCCTCTGCAACTCCGACATCGTATCCGGCAAGGTGG	420
ProSerHisProGlnAspAlaPheCysAsnSerAspIle <u>ValIleArgAlaLysValVal</u>	24
TGGGAAAGAAGCTGGTAAAGGAGGGCCCTCGGCACGCTGGTCTACACCATAAGCAGA	480
GlyLysLysLeuValLysGluGlyProPheGlyThrLeuVal <u>TyrThrIleLysGlnMet</u>	44
TGAAGATGTACCGAGGCTCACCAAGATGCCCATGTGCACTACATCCATAACGGAAAGCTT	540
LysMetTyrArgGlyPheThrLysMetProHisValGlnTyrIleHisThrGluAlaSer	64
CCGAGAGTCTGTGGCTTAAGCTGGAGGTCAACAAGTACCACTGCTGCTGACAGGTC	600
GluSerLeuCysGlyLeuLysLeuGluValAsnLysTyrGlnTyrLeuLeuThrGlyArg	84
GCGTCTATGATGGCAAGATGTACACGGGCTGTGCAACTTCGTGGAGAGGTGGACCAGC	660
ValTyrAspGlyLysMetTyrThrGlyLeuCysAsnPheValGluArgTrpAspGlnLeu	104
TCACCCCTCTCCAGCGCAAGGGCTGAACATCGTATCACCTGGTTGTAAGTCAAGA	720
ThrLeuSerGlnArgLysGlyLeuAsnTyrArgTyrHisLeuGlyCysAsnCysLysIle	124
TCAAGTCCTGCTACTACCTGCCCTGCTTGTGACTTCCAAGAACGAGTGTCTGGACCG	780
LysSerCysTyrTyrLeuProCysPheValThrSerLysAsn <u>GluCysLeuTrpThrAsp</u>	144
ACATGCTCTCCAATTTCGGTTACCCCTGGCTACCAGTCAAACACTACGCCCTGCATCCGGC	840
<u>Met</u> LeuSerAsnPheGlyTyrProGlyTyrGlnSerLysHisTyrAlaCysIleArgGln	164
AGAAGGGCGGCTACTGCAGCTGGTACCGAGGATGGGCCCCCGATAAAAGCATCATCA	900
LysGlyGlyTyrCysSerTrpTyrArgGlyTrpAlaProProAspLysSerIleIleAsn	184
ATGCCACAGACCCCTGAGGCCAGACCTGCCACCTCACTTCCCTCCCTCCGCTGA	960
AlaThrAspProEnd	188
GCTTCCCTGGACACTAACTCTCCAGATGATGACAATGAAATTAGTGCCTGTTTCTT	1020
GCAAATTAGCACTGGAACATTAAAGAAAGGTCTATGCTGTCATATGGGGTTATTGG	1080
GAACATCCTCTGGCCCCACCCCTGCCCTCTTTGGTTTGACATCATTCAATTCCA	1140
CCTGGGAATTCTGGTGCCATGCCAGAAAGAATGAGGAACCTGTATTCCCTTTCGTG	1200
ATAATATAATCTCTATTAGGAAAAAAAAAAAAAAA	1240

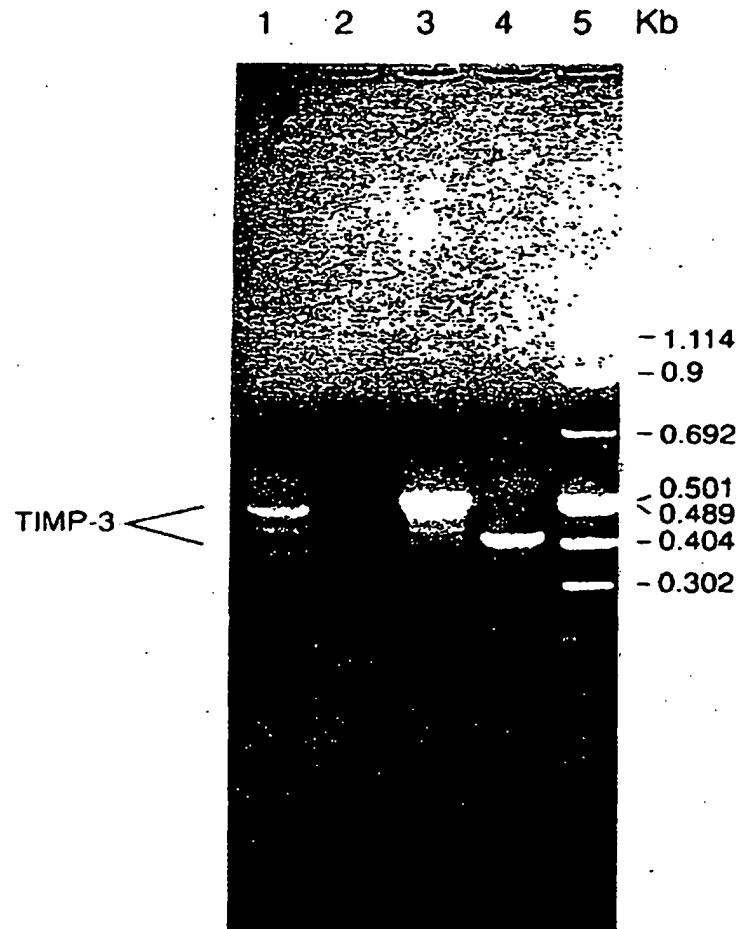


FIG. 2

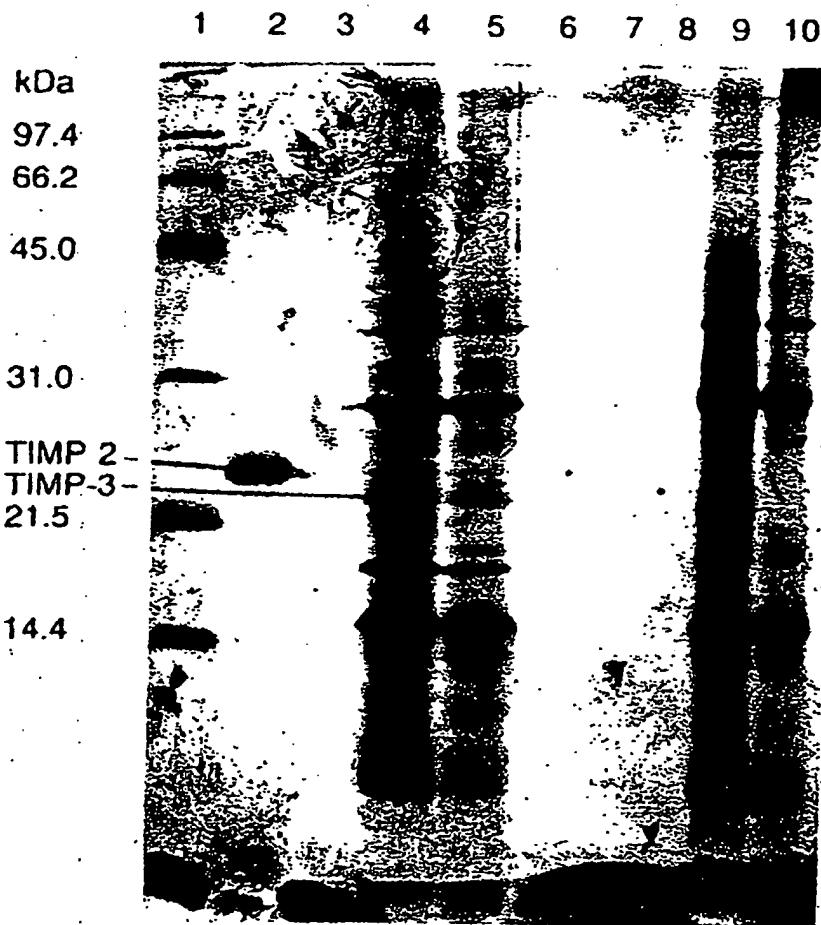


FIG. 3

FIG. 4

-23	MAPPFAPM ASCILLLWL TAPSRA[CTCV PPHPQTAPCN SDV VIRAKFV MAPPEPL ASCILLLWL IAPSRA[CTCV PPHPQTAFCN SDLVIRAKFV MAPLAAL ASSMLLLWL VAPSRA[CTCV PPHPQTAFCN SDLVIRAKFV MAPPASL ASCILLLWL IASSKA[CSCA PPHPQTAFCN SDLVIRAKFM Bovine TIMP-2 MGAAARSLPL AFCLLLLGL LPRADA[CSCS PVHPQQAFCN ADIVIRAKAV Human TIMP-2 MGAAARTLRL ALGLLLLTL LRPADA[CSCS PVHPQQAFCN ADVVIRAKAV Mouse TIMP-2 MGAAARSLRL ALGLLLLASL VRPADA[CSCS PVHPQQAFCN ADVVIRAKAV Chick TIMP-3 MTAWLGFLAV FLCWSLRLD VAEA[CTCV PIHPQDAPCN SDIVIRAKVV Human TIMP-3 MTPWLGLI.V LLGSWSLGDW GAEA[CTCS PSHPQDAPCN SDIVIRAKVV	1	24
25 ***			
Bovine TIMP-1 Human TIMP-1 Rabbit TIMP-1 Mouse TIMP-1 Bovine TIMP-2 Human TIMP-2 Mouse TIMP-2 Chick TIMP-3 Human TIMP-3	GTAEVNETAL Y.....QR YEIKMTKMFK GPSALRDAPD IRFIYTPAME GTPEVNQTTL Y.....QR YEIKMTKMFK GFQALGDAAD IRFVYTPAME GAPEVNHTTL Y.....QR YEIKMTKMFK GFDALGHATD IRFVYTPAME GSPEINETTL Y.....QR YKIKMTKMLK GFKA VGNNAAD IRYAYTPVME NKKEVDSGND IYGNPIKRIQ YEIKQIKMFK GPDQ....D IEFIYTAPAA SEKEVDSGND IYGNPIKRIQ YEIKQIKMFK GPEK....D IEFIYTAPSS SEKEVDSGND IYGNPIKRIQ YEIKQIKMFK GPDK....D IEFIYTAPSS GKKLMKDQ.. .PFGTMR YTVKQMCKMYR GFQIM...PH VQYIYTEASE GKKLVKEG.. .PFGTLV YTIKQMCKMYR GFTKM...PH VQYIYTEASE	65	
66 ***			
Bovine TIMP-1 Human TIMP-1 Rabbit TIMP-1 Mouse TIMP-1 Bovine TIMP-2 Human TIMP-2 Mouse TIMP-2 Chick TIMP-3 Human TIMP-3	SVCGYFHSQ NRSEEFLIAG QLSNGHLHIT TCSFVAPWNS MSSAQRRGFT SVCGYFHRSH NRSEEFLIAG KLQDGGLLHIT TCSFVAPWNS LSLAQRRGFT SVCGYSHKSQ NRSEEFLIAG QLRNGLLHIT TCSFVVPWNS LSFSQRSGFT SLCGYAHKSQ NRSEEFLITG RLRNGNLHIS ACSFLVPWRT LSPAQQRRAFS AVCGVSLSDIG GKKEYLIAGK AEGNGNMHIT LCDFIVPWDT LSATQKKSLSN AVCGVSLDVG GKKEYLIAGK AEGDGKMHIT LCDFIVPWDT LSITQKKSLSN AVCGVSLDVG GKKEYLIAGK AEGDGKMHIT LCDFIVPWDT LSITQKKSLSN SLCGVKLEV. NKYQYLITGR VY.EGKVYITG LCNWYEKWDR LTLSQRKGGLN SLCGLKLEV. NKYQYLLTGR VY.DGKMYTG LCNFVERWDQ LTLSQRKGGLN	113	
114			
Bovine TIMP-1 Human TIMP-1 Rabbit TIMP-1 Mouse TIMP-1 Bovine TIMP-2 Human TIMP-2 Mouse TIMP-2 Chick TIMP-3 Human TIMP-3	KTYAAGCEEC TVFPCSSIPC KLQSDTHCLW TDQLLTGSDK GFQSRHLACI KTYTVGCEEC TVFPCLSIPC KLQSGTHCLW TDQLLQGSEK GFQSRHLACI KTYAACGDMC TVFACASIPC HLESDTTHCLW TDSSL.GSDK GFQSRHLACI KTYSAGCCVC TVFPCLSIPC KLESDTTHCLW TDQVLVGSE. DYQSRHFACI HRYQMGCE.C KITRCPMIPC YISSPDECLW MDWVTEKNIN GHQAKFFACI HRYQMGCE.C KITRCPMIPC YISSPDECLW MDWVTEKNIN GHQAKFFACI HRYQMGCE.C KITRCPMIPC YISSPDECLW MDWVTEKSIN GHQAKFFACI HRYHLGGC.C KIRPCYYLPC FAT9KNECIW TDMLSNGHS GHQAKHYACI YRYHLGCC.C KIKSCYYLPC FVTSKNECLW TDMLSNGYP GYQSKHYACI	162	
163 188			
Bovine TIMP-1 Human TIMP-1 Rabit TIMP-1 Mouse TIMP-1 Bovine TIMP-2 Human TIMP-2 Mouse TIMP-2 Chick Timp-3 Human TIMP-3	PREPGLCTWQ SLRAQMA... PREPGLCTWQ SLRSQIA... PQEPEGLCAWE SLRPRKD... PRNPGLCTWR SLGAR... KRSRGSCAWY RGAAPPKQEF LDIEDP KRSRGSCAWY RGAAPPKQEF LDIEDP KRSRGSCAWY RGAAPPKQEF LDIEDP QRVEGYCSWY RGWAPPDKTI INATDP RQKGGYCSWY RGWAPPDKSI INATDP		

FIG. 5

TIMP-3	1	MTPWLGLI.VLLGSWSLGDWGAECTCSPSHQD AFCNSDIVIRAKVVGK	49
	: :: :.- :- : : : :		
ChIMP-3	1	MTAWLGFLAVFLCSWLSRLDVAEACTCVP IHPQD AFCNSDIVIRAKVVGK	50
	: :: :.- :- : : : :		
TIMP-3	50	KLVKEGPGFTLVYT IKQM KMYRGFTKMPHVQYI HTEASES LCGKLKLEV NK	99
	: :: :.- :- : : : :		
ChIMP-3	51	KLMKDGPFGTMRYTVKQ M KMYRGFQIMPHVQYI YTEASES LCGVKLEV NK	100
	: :: :.- :- : : :		
TIMP-3	100	YOYLLTGRVYDGKMYTGLCNFVERWDQLTLSQRKGLN YRYHLCNC KIKS	149
	: :: :.- :- : : : :		
ChIMP-3	101	YQYLITGRVYEGKVTGLCNWYEKWDRLTLSQRKGLN HRYHLCGC KIRP	150
	: :: :.- :- : : :		
TIMP-3	151	CYYLPCFVTSKN ECLWT DMLS NFGYPGYQSKHYACI RQKGGYCSWY RGWA	200
	: :: :.- :- : : : :		
ChIMP-3	150	CYYLPCFATSKNECI WTDMLS NFGHSGHQAKHYACI QRVE GYCSWY RGWA	199
	: :: :.- :- : : :		
TIMP-3	200	PPDKSIINATDP	211
	: :: :.- :-		
ChIMP-3	201	PPDKTIINATDP	212

FIG. 6A

TIMP-3	151	CGCCGGAGGCCAAGGTTGCCCGCACGGCCGGCGAGCAGCTCGG	200
ChIMP-3	1CGCGAGAGAGAGGGCGGTGTGAGGAGGGAGCGAGCAGCG	42
TIMP-3	201	GCTGCAGCAGCCCCGCCGGCGCGCACGGCAACTTGGAGAGGGCGAGC	250
ChIMP-3	43	AACAGGCGAGGCTCGAGTTAGGCGAACAGAACAGCGGCTGCAGCTCGAAG	92
TIMP-3	251	AGCAGCCCCGGCAGCGGCGGCAGCAGCGCAATGACCCCTGGCTCGG..	298
Chimp-3	393	CGCACCCCCGGG.....GCAGGCAGCATGACGGCGTGGCTCGGCT	131
TIMP-3	299	.GCTCATCGTGCCTGGCAGCTGGAGCCTGGGGACTGGGCGCCGAG	347
ChIMP-3	132	TCCTCGCCGTGTCCTGTGCAGCTGGAGCCTGCGGGACCTGGTGGCGGAG	181
TIMP-3	348	GCGTGCACATGCTGCCAGCCACCCCCAGGACGCCCTCTGCAACTCCGA	397
ChIMP-3	182	GCGTGCACATTGCGTCCCCATCCACCCGCAAGGACGCGTTCTGCAACTCCGA	231
TIMP-3	398	CATCGT GATCCGGGCCAAGGTGGTGGGAAGAAGCTGGTAAAGGAGGGGC	447
Chimp-3	232	CATCGT GATCCGTGCTAAAGTTGTGGGAAGAAGCTCATGAAAGATGGAC	281
TIMP-3	448	CCTTCGGCACGCTGGTCTACACCATCAAGCAGATGAAGATGTACCGAGGC	497
ChIMP-3	282	CATTGGAACAATGCGATACACAGTCAGCAAGCAGATGAAGATGTACAGGGC	331
TIMP-3	498	TTCACCAAGATGCCCATGTGCAGTACATCCATACGGAAGCTTCCGAGAG	547
ChIMP-3	332	TTCCAGATAATGCCACACGTTAGTACATCTACACAGAACGCTCAGAGAG	381
TIMP-3	548	TCTCTGTGGCCTTAAGCTGGAGGTCAACAAGTACCAAGTACCTGCTGACAG	597
ChIMP-3	382	TCTTTGTGGTGTGAAACTGGAGGTCAACAAATACCAGTATCTGATTACAG	431
TIMP-3	598	GTCGCGTCTATGATGGCAAGATGTACACGGGGCTGTGCAACTCGTGGAG	647
ChIMP-3	432	GCCGCGTGTACGAAGGGAAGGTTACACTGGCCTGTGCAATTGGTATGAG	481
TIMP-3	648	AGGTGGGACCAGCTACCCCTCTCCAGCGCAAGGGCTGAACATCGGTA	697
ChIMP-3	482	AAATGGGACCGACTGACTCTGTCCCAGCGTAAAGGACTGAATCATCGTTA	531
TIMP-3	698	TCACCTGGGTTGTAAGTCAAGATCAAGTCTGCTACTACCTGCCTTGCT	747
ChIMP-3	532	TCATCTGGGCTGTGGATGCAAGATTGGCCCTGCTACTATTGCCCCGT	581

FIG. 6B

FIG. 7A

TIMP-3	282	ATGACCCCTGGCTGGGCTCAT...CGTGCCTGGCAGCTGGAGCCT	328
ChIMP-3	113	ATGACGGCGTGGCTGGCTTCCTGCCGTGTTCTGTGCAGCTGGAGCCT	162
TIMP-3	329	GGGGGACTGGGGCGCCGAGGCGTGCACATGCTGCCAGCCACCCCCAGG	378
ChIMP-3	163	GCGGGACCTGGTGGCGGAGGCGTGCACTTGCGTCCCCATCCACCCGCAGG	212
TIMP-3	379	ACGCCTCTGCAACTCGACATCGTATCCGGCAAGGTGGTGGGAAGG	428
ChIMP-3	213	ACCGCGTCTGCAACTCGACATCGTATCCGTGCTAAAGTTGTGGGAAGG	262
TIMP-3	429	AAGCTGGTAAAGGAGGGGCCCTCGGCACGCTGGTCTACACCATAAGCA	478
ChIMP-3	263	AAGCTCATGAAAGATGGACCATTGGAACAATGCGATAACACAGTCAGCAAGCA	312
TIMP-3	479	GATGAAGATGTACCGAGGCTTCACCAAGATGCCCATGTGCAGTACATCC	528
ChIMP-3	313	GATGAAGATGTACAGGGCTTCAGATAATGCCACACGTTCAAGTACATCT	362
TIMP-3	529	ATACGGAAGCTTCCGAGAGTCTCTGTGGCCTTAAGCTGGAGGTCACAAAG	578
ChIMP-3	363	ACACAGAAGCCTCAGAGAGTCTTGTGGTGTGAAACTGGAGGTCACAAA	412
TIMP-3	579	TACCACTGCTGACAGGTGCGCTATGATGGCAAGATGTACACGGG	628
ChIMP-3	413	TACCACTGATTACAGGCCGCGTGTACGAAGGGAAAGGTTACACTGG	462
TIMP-3	629	GCTGTGCAACTCGTGGAGAGGTGGGACCAAGCTCACCTCTCCAGCGCA	678
ChIMP-3	463	CCTGTGCAATTGGTATGAGAAATGGGACCGACTGACTCTGTCCAGCGTA	512
TIMP-3	679	AGGGGCTGAACTATCGGTATCACCTGGTTGTAACGTCAAGATCAAGTCC	728
ChIMP-3	513	AAGGACTGAATCATCGTTATCATCTGGCTGTGGATGCAAGATTGGCCC	562
TIMP-3	729	TGCTACTACCTGCCCTGCTTGTGACTTCCAAGAACGAGTGTCTCTGGAC	778
ChIMP-3	563	TGCTACTATTGCCCTGCTTGTGCCACCTCCAAGAACGAGTGCATTGGAC	612
TIMP-3	779	CGACATGCTCTCCAATTTCGGTTACCCCTGGCTACCAAGTCCAAACACTACG	828
ChIMP-3	613	AGACATGCTCTCCAACCTCGGCCACTCAGGACACCAAGCGAACGACTATG	662
TIMP-3	829	CCTGCATCCGGCAGAAGGGCGGCTACTGCAGCTGGTACCGAGGATGGGCC	878
ChIMP-3	663	CCTGCATCCAGAGGGTGGAAAGGTTACTGCAGCTGGTATAGAGGATGGCG	712

FIG. 7B

FIG. 8

TIMP-3	1	MTPWLGLIVLLGSWSLGDWGAECTCSPSHPQDAFCNSDIVIRAK	45
		. : : : . . : . : . :	
TIMP-2	1	MGAAARTLRLALGLLLLATLL..RPADACSCSPVHPQQAFCNADVIRAK	48
		.: . . . : . : : : . .::: . .: ..	
TIMP-3	46	VVGKKLVKEG.....PFCTLVYTIKQMKMYRGFTKMPHVQYIHTEASES	89
		.: : . . : . : : : . .::: . .: ..	
TIMP-2	49	AVSEKEVDGNDIYGNPIKRIQYEIKQIKMFKGPEK..DIEFIYTAPSSA	96
		.: : : : . : : : : . . - : .	
TIMP-3	90	LCGLKLEV.NKYQYLLTGRVY.DGKMYTGLCNFVERWDQLTLSQRKGLNY	137
		: : : : : . : : : : . . - : .	
TIMP-2	97	VCGVSLDVGGKKEYLIAGKAEGDGKMHTLCDFIVPWDLSTTQKKSLNH	146
		.: : : : : . : : : : . . - : .	
TIMP-3	138	RYHLCNCIKSCYYLPCFVTSKNECLWTDMLSNFGYPGYQSKHYACIRQ	187
		. : : : : . : : : : . . - : .	
TIMP-2	147	RYQMGCECKITRCPMIPCYISSPDECLWMDWVTEKNINGHQAKFFACIKR	196
		.: : : : : . : : : : . . - : .	
TIMP-3	188	KGGYCSWYRGWAPPDKSIINATDP	211
		. . . : ::: .	
TIMP-2	197	SDGSCAWYRGAAPPKQEFLDIEDP	220

FIG. 9A

TIMP-3	1	GGCGGCGGGCGCTCAGACGGCTTCTCCTCCCTCTTGCTCCTCCAAGCT	50
TIMP-2	1GGGGCCGCCGAGAGCCGCAGGCCGCTGCCCGCCGCCCCCACC	45
TIMP-3	51	CCTGCTCCTTCGCCGGAGCCCGCCGCCAGTCCTGCGCCAGGCCAG 100	
TIMP-2	46	CCGCCGCCCGCCCGGAATTGCGCCCGGCCCTCCCTCGGCCCGCC 95	
TIMP-3	101	GCAGCCTCGCTGCCGCCATCCCCTCCGCCGGCACTCGGAGGGCAGCG 150	
TIMP-2	96	GAGACAAAGAGGAGAGAAAGTTGCGCCGGAGCCGGCAGGTGAGGAGG 145	
TIMP-3	151	CGCCGGAGGCCAAGGTTGCCCGCACGGCCCGGCCAGCGAGCTCGG 200	
TIMP-2	146	GTGAGCCGCCGGAGGGGCCCTGGCCCGCTCAGCCCCCGCCCG 195	
TIMP-3	201	GCTGCAGCAGCCCGCCG.GCGGCGCGCACGGCAACTTGGAGAGG.... 245	
TIMP-2	196	GCCCCCAGCCCGCCGCCGAGCAGGCCCGAACCCCCCAGCGCCGGCCC 245	
TIMP-3	246CGAGCAGCAGCCCGGCAGCGGCCAGCAGGGCAATGACCCCTT 291	
TIMP-2	246	CGCCCGCCAGCCCCCGGCCATGGCGCCGCCACCCCTGC 295	
TIMP-3	292	GGCTGGGCTCATCGTGTCTGGCAGCTGGAGCCTGGGGACTGGGC 341	
TIMP-2	296	GGCTGGCGCTCGGCCTCTGCTG.....CTGGGACGCTGTTGCCCG 339	
TIMP-3	342	GCCGAGGCCTGCACATGTCGCCAGCCACCCCCAGGACGCCCTCTGCAA 391	
TIMP-2	340	GCCGACGCCCTGCAGCTGCTCCCCGGTGCACCGAACAGGCCTTGCAA 389	
TIMP-3	392	CTCCGACATCGTGTACCGGGCCAAGGTGGTGGGAAGAAGCTGGTAAAGG 441	
TIMP-2	390	TGCAGATGTAGTGTACAGGGCAAAGCGGTCACTGAGAAGGAAGTGGACT 439	
TIMP-3	442	AGGG.....GCCCTCGGCACGCTGGTCTACACCATC 473	
TIMP-2	440	CTGGAAACGACATTATGGCAACCCATCAAGAGGATCCAGTATGAGATC 489	
TIMP-3	474	AAGCAGATGAAGATGTACCGAGGCTCACCAAGATGCCCATGTGCAGTA 523	
TIMP-2	490	AAGCAGATAAAGATGTTCAAAGGGCTGAGAAG.....GATATAGAGTT 533	
TIMP-3	524	CATCCATACGGAAGCTCCGAGAGTCTCTGTGGCCTTAAGCTGGAGGT.. 571	
TIMP-2	534	TATCTACACGGCCCCCTCCTCGGCAGTGTGTGGGTCTCGCTGGACGTTG 583	

FIG. 9B

TIMP-3	572	.CAACAAGTACCAAGTACCTGCTGACAGGTGCGCTATG...	ATGGCAAG	617	
TIMP-2	584	GAGGAAGAAGGAATATCTCATTGCA	AGGAAAGGCCGAGGGGGACGGCAAG	633	
TIMP-3	618	ATGTACACGGGCTGTGCAACTTCGTGGAGAGGTGGACCAGCTCACCT	667		
TIMP-2	634	ATGCACATCACCCCTGTGACTTCATCGTGCCTGGACACCTGAGCAC	683		
TIMP-3	668	CTCCCAGCGCAAGGGGCTGA	ACTATCGGTATCACCTGGGTGTA	ACTGCA	717
TIMP-2	684	CACCCAGAAGAAGAGCCTGA	ACCACAGGTACCA	GATGGGTCGAGTGCA	733
TIMP-3	718	AGATCAAGTCTGCTACTACCTGCCTTGCTTGACTTCCAAGAACGAG	767		
TIMP-2	734	AGATCACCGCCTGCCCATGATCCCCTGCTACATCTCC	CCCCGGACGAG	783	
TIMP-3	768	TGTCTCTGGACCGACATGCTCTCCAATT	CGTTACCC	TGGTACCA	817
TIMP-2	784	TGCCTCTGGATGGACTGGGT	CACAGAGAACATCAAC	GGGCCACCAGGC	833
TIMP-3	818	CAAACACTACGCC	TGCATCCGGCAGAAGGGCGGCTACTGCAGCTGGTACC	867	
TIMP-2	834	CAAGTTCTCGCC	TGCATCAAGAGAACGTGACGGCT	CCGTGGTACC	883
TIMP-3	868	GAGGATGGGCCCCCGGATAAAAGCATCATCA	ATGCCACAGACCCCTGA	917	
TIMP-2	884	GGGGCCGGCGCCCCCAAGCAGGAGTTCTGACATCGAGGACCCATAA	933		
TIMP-3	918	GCGCCAGACCC	TGCCACCTCACTTCC	CTCCCTCCGCTGAGCTCCC	967
TIMP-2	934	GCAGGCCTCCAAC	GGCCCTGTTGCAACTGCA	AAAAGCCTCCAAGGGT	983
TIMP-3	968	TTGGACACTAACTCTCCCAGATGATGACAATGAAATTAGTGCCTGTTT	1017		
TIMP-2	984	TTCGACTGGTCCAGCTCTGACATCC	CTCCCTGGAAA.....	CAGCATGA	1027
TIMP-3	1018	CTTGCAAATTAGC	ACTTGGAACATTAAAGAAAGGTCTATGCTGTCATA	1067	
TIMP-2	1028	ATAAAACACTCATCCC	ATGGTCAAATTAAATATG.....	1062

FIG. 10A

FIG. 10B

TIMP-3	734	CTACCTGCCTTGCTTTGTGACTTCCAAGAACGAGTGTCTCTGGACCGACA	783
TIMP-2	750	CATGATCCCGTGCTACATCTCCTCCCCGACGAGTGCCTCTGGATGGACT	799
TIMP-3	784	TGCTCTCCAATTTCGGTTACCCTGGCTACCAGTCCAAACACTACGCCCTGC	833
TIMP-2	800	GGGTCACAGAGAAGAACATCAACGGGCACCAGGCCAAGTTCTCGCCTGC	849
TIMP-3	834	ATCCGGCAGAAGGGCGGCTACTGCAGCTGGTACCGAGGATGGGCCCCCC	883
TIMP-2	850	ATCAAGAGAAGTGACGGCTCCTGTGCGTGGTACCGCGGGCGCGGGCGCCCCC	899

FIG. 11

TIMP-3 1 MAPFEPLASGILLLWLIAPSR...ACTCVPPHPQTAFCNSDLVIRAKFV 47
 | .: .| | :|| .: ... |||||.|||.||||||:|||||.|||:
 TIMP-1 1 MTPWLGL...IVLLGSWSLGDWGAEACTCSPSHPQDAFCNSDIVIRAKVV 47
 | . .| .: .| .|||:||| .: .: .: .| .|||:
 TIMP-3 48 GTPEVNQTTL.YQRYEIKMTKMYKGFQALGDAADIRFVYTPAMESVCGYF 96
 | . .| .: .| .|||:||| .: .: .: .| .|||:
 TIMP-1 48 GKKLVKEGPFGTLVYTICKQMKMYRGFTKM...PHVQYIHTEASESLCGL. 93
 | . .| .: .| .|||:||| .: .: .| .|||:
 TIMP-3 97 HRSHNRSEEFILAGKLQDGLLHITTCSFVAPWNSLSLAQRGGFTKTYTVG 146
 | . .| .: .| .|||:||| .: .: .| .|||:
 TIMP-1 94 KLEVNKYQYLLTGRVYDGKMYTGLCNFVERWDQLTLSQRKGGLNYRYHLG 142
 | . .| .: .| .|||:||| .: .: .| .|||:
 TIMP-3 147 CEECTVFPCLSI PCKLQSGTHCLWTDQLLQGSEKGFQSRHLACLPREPGL 196
 | . .| .: .| .|||:||| .: .: .| .|||:
 TIMP-1 143 C.NCKIKSCYYLPCFVTSKNECLWTDMLSNFGYPGYQSKHYACIRQKGGY 191
 | . .| .: .| .|||:
 TIMP-3 197 CTWQSLRSQIA..... 207
 | . .| .: .| .|||:
 TIMP-1 192 CSWYRGWAPPDKSIINATDP 211

FIG. 12A

TIMP-3	201	GCTGCAGCAGCCCCGCCGGCGCGCACGGCAACTTGGAGAGGGCGAGC	250
TIMP-1	1AGGGCCTTAGCGTGCCGATGCCGAGATC	31
TIMP-3	251	AGCAGCCCCGGCAGCGCGGGCAGCAGCGGCAATGACCCCTGGCTCGGGC	300
TIMP-1	32	CAGCGCCCAGAGAGACACCAGAGAACCCACCATGGCCCCCTTGAGCCCC	81
TIMP-3	301	TCATCGTGCTCCTGGCAGCTGGAGGCCTGGGGACTGGGGGCCGAGGCG	350
TIMP-1	82	TGGCTCTGGCATCCTGTTGCTGTGGCTGATAGCCCCCAGCAGGGCC	131
TIMP-3	351	TGCACATGCTCGCCAGCCACCCCCAGGACGCCTCTGCAACTCCGACAT	400
TIMP-1	132	TGCACCTGTGTCCCACCCACAGACGGCCTCTGCAATTCCGACCT	181
TIMP-3	401	CGTGATCCGGCCAAGGTGGTGGGAAGAAGCTGGTAAAGGAGGGCCCT	450
TIMP-1	182	CGTCATCAGGCCAACGTTCTGTGGGACACCAGAAGTCAACCAGACCACCT	231
TIMP-3	451	TCGGCACCG.....TGGTCTACACCATAAGCAGATGAAGATGTACCGA	494
TIMP-1	232	TATACCAGCGTTATGAGATCAAGATGACCAAGATGTATAAAGGGTTCAA	281
TIMP-3	495	GGCTTCACCAAGATGCCCATGTGCAGTACATCCATAACGGAAAGCTTCCGA	544
TIMP-1	282	GCCTTAGGGATGCCGCTGACATCCGGTCTGTACACCCCCGCCATGGA	331
TIMP-3	545	GAGTCTCTGTGG.....CCTTAAGCTGGAGGTCAACAAGTACCACTGACC	588
TIMP-1	332	GAGTGTCTCGGATACTTCCACAGGTCCCACAACCGCAGCGAGGAGTTTC	381
TIMP-3	589	TGCTGACAGGTGCGCTATGATGGCAAGATGTACACGGGCTGTGCAAC	638
TIMP-1	382	TCATTGCTGGAAAATGCAGGATGGACTCTTGCACATCACTACCTGCAGT	431
TIMP-3	639	TTCTGGAGAGGTGGACCAGCTCACCTCTCCAGCGCAAGGGGCTGAA	688
TIMP-1	432	TTCTGGCTCCCTGGAACAGCCTGAGCTAGCTCAGCGCCGGGCTTCAC	481
TIMP-3	689	CTATCGGTATCACCTGGGTGT...AACTGCAAGATCAAGTCTGCTACT	735
TIMP-1	482	CAAGACCTACACTGTTGGCTGTGAGGAATGCACAGTGTTCCTGTTAT	531
TIMP-3	736	ACCTGCCTTGCTTGTGACTTCAAGAACCGAGTGTCTCTGGACCGACATG	785
TIMP-1	532	CCATCCCCCTGCAAACCTGCAGAGTGGCACTATTGCTGTGGACGGACAG	581

FIG. 12B

FIG. 13

TIMP-3	347	GGCGTGCACATGCTGCCAGCCACCCCCAGGACGCCCTCTGCAACTCCG	396
TIMP-1	128	GGCCTGCACCTGTGTCCCACCCCACCCACAGACGCCCTCTGCAATTCCG	177
TIMP-3	397	ACATCGTGATCCGGGCCAAGGTGGTGGGGAAAGAAGCTGGTAAAGGAGGGG	446
TIMP-1	178	ACCTCGTCATCAGGGCCAAGTTCGTGGGACACCAGAACTAACCAAGAGACC	227
TIMP-3	447	CCCTTCGGCACGCTGGTCTACACCATCAAGCAGATGAAGATGTACCGAGG	496
TIMP-1	228	ACCTTATA...CAGCGTTATGAGATCAAGATGACCAAGATGTATAAAGG	274
TIMP-3	497	CTTC 500	
TIMP-1	275	GTTC 278	

2.5 4.4 kb
1 1



MW
Hs 68
Hs 27
A172
Hs 578T
Hs 294T
A498
293
SK-NEP-1
WI 38
WI-26 VA4
CCD-11Lu
MDA-MB-231
DU4475
BT-474
Caov-3
SK-OV-3
Hep G2
SK-Hep-1

FIG. 14A

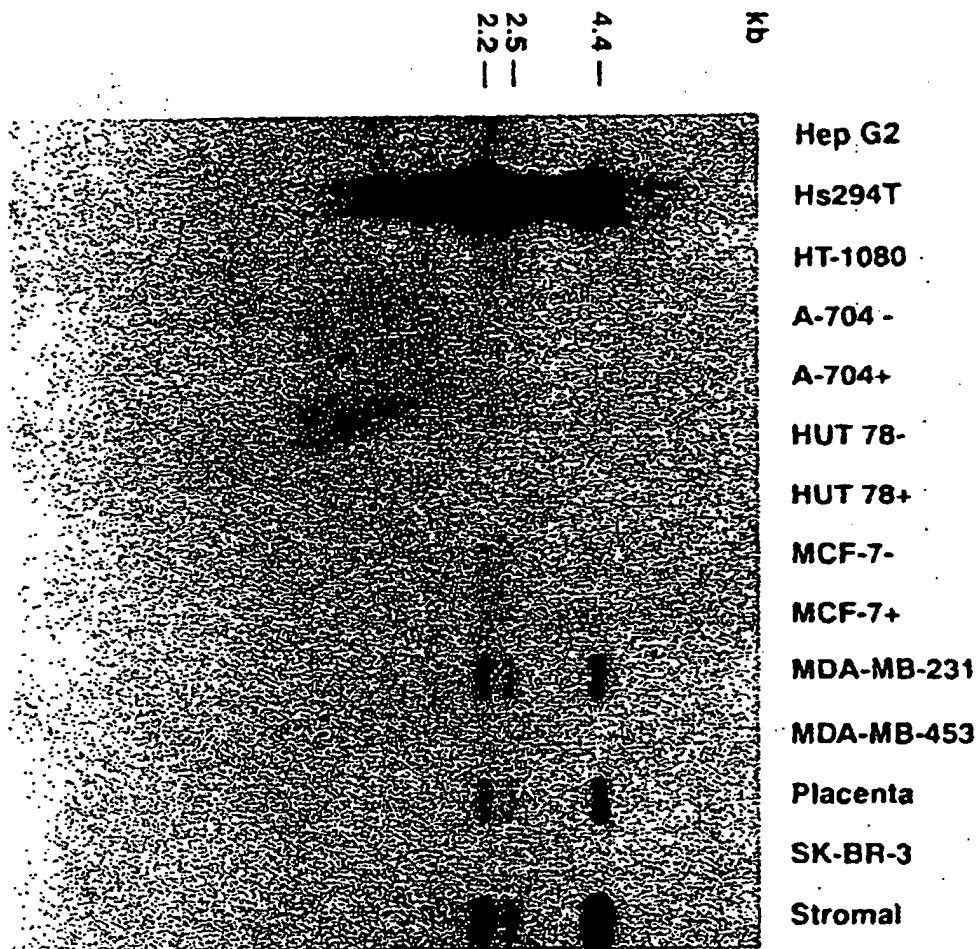


FIG. 14B

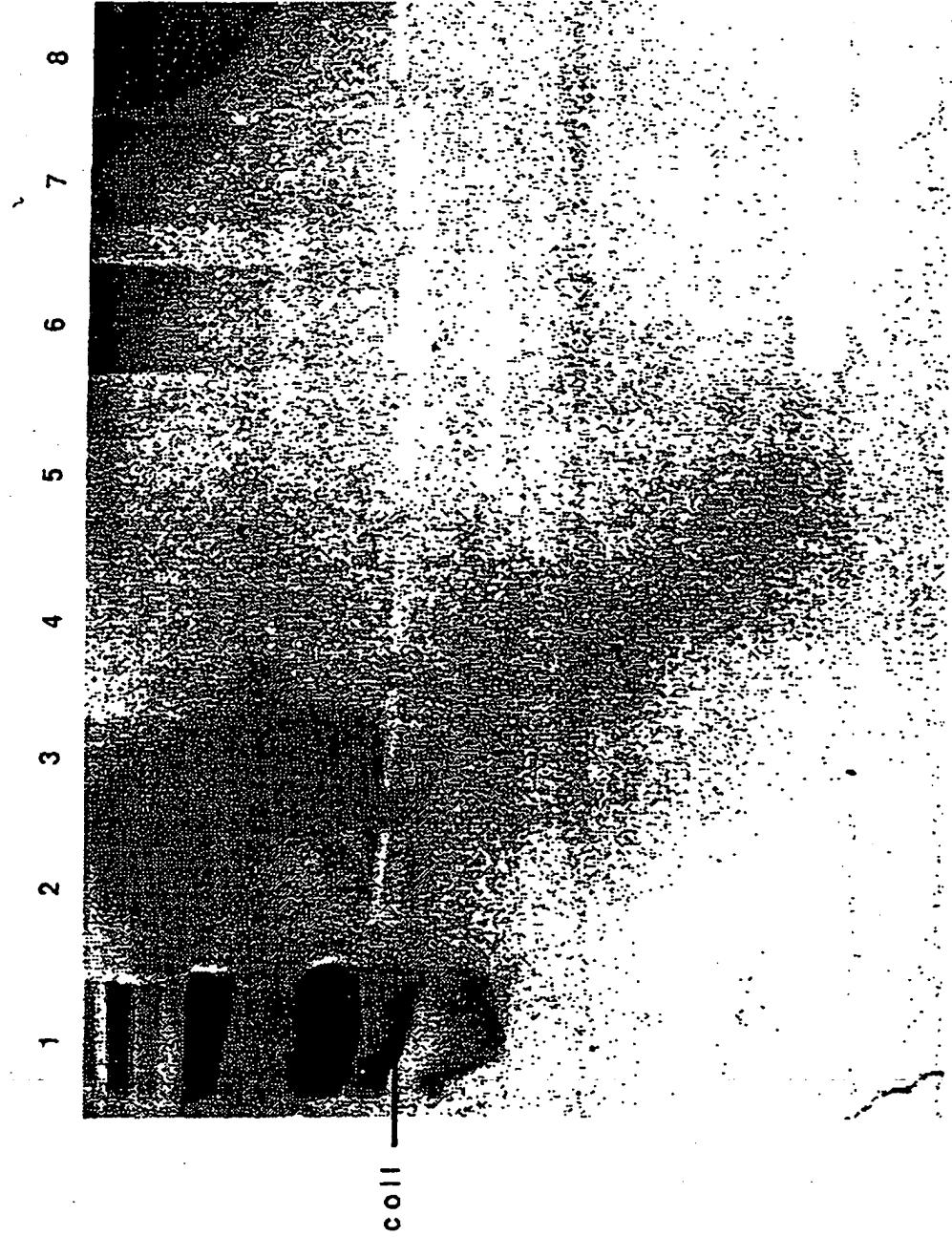


FIG. 15

FIG. 16A

TIMP3clone7	GGCGGGGGGGGCTCAGACGGCTCTCCCTCCCTTGCTCCCAAGCTCCTGCTCCCT	60
TIMP3clone2	
TIMP3HCM-3	
TIMP3PCR29	
TIMP3clone7	CGCCGGGAGCCCGCCCGCCGAGTCCTGCGCCAGGCCGAGGCAGCCTCGCTGCGCCCCAT	120
TIMP3clone2	
TIMP3HCM-3	
TIMP3PCR29	
TIMP3clone7	CCCGTCCC GCCGGGACTCGGAGGGAGCGCCGGAGGCCAAGGTTGCCCGCACGGCC	180
TIMP3clone2	
TIMP3HCM-3	
TIMP3PCR29	
TIMP3clone7	CGCGGGCGAGCGAGCTCGGCTGCCAGCAGCCCCGCCGGCGCGCACGGCAACTTTGG	240
TIMP3clone2	
TIMP3HCM-3	
TIMP3PCR29	
TIMP3clone7	AGAGGCAGCAGCAGCCCCGGCAGCGGGCAGCAGCGGAATGACCCCTGGCTCGGGC	300
TIMP3clone2	
TIMP3HCM-3	
TIMP3PCR29	
TIMP3clone7	MetThrProTrpLeuGlyL euIleValLeuLeuGlySerTrpSerLeuGlyAspTrpGlyAlaGluAlaCysThrCysS TCATCGTGCTCTGGGCAGCTGGAGCCCTGGGGACTGGGGCGCCGAGGCGTGCACATGCT	-16 360
TIMP3clone2 CAGGAGCTGGGGACTGGGGCGCCGAGGCGTGCACATGCT Arg	40
TIMP3HCM-3	
TIMP3PCR29	
TIMP3clone7	erProSerHisProGlnAspAlaPheCysAsnSerAspIleValIleArgAlaLysValV CGCCCAGCCACCCCCAGGACGCCCTCTGCAACTCCGACATCGTGATCCGGGCCAAGGTGG	25 420
TIMP3clone2 CGCCCAGCCACCCCCAGGACGCCCTCTGCAACTCCGACATCGTGATCCGGGCCAAGGTGG	100
TIMP3HCM-3	
TIMP3PCR29	

FIG. 16B

TIMP3clone7	a1GlyLysLysLeuValLysGluGlyProPheGlyThrLeuValTyrThrIleLysGlnM TCGGGAAGAAGCTGGTAAAGGAGGGCCCTTCGGCACGCTGGCTACACCATCAAGCAGA	45 480
TIMP3clone2	TCGGGAAGAAGCTGGTAAAGGAGGGCCCTTCGGCACGCTGGCTACACCATCAAGCAGA	160
TIMP3HCM-3GGGAAGAAGCTGGTAAAGGAGGGCCCTTCGGCACGCTGGCTACACCATCAAGCAGA	58
TIMP3PCR29	
TIMP3clone7	etLysMetTyrArgGlyPheThrLysMetProHisValGlnTyrIleHisThrGluAlaS TGAAGATGTACCGAGGCTTCACCAAGATGCCCATGTGCAGTACATCCATACGGAAGCTT	65 540
TIMP3clone2	TGAAGATGTACCGAGGCTTCACCAAGATGCCCATGTGCAGTACATCCACACGGAAGCTT	220
TIMP3HCM-3	TGAAGATGTACCGAGGCTTCACCAAGATGCCCATGTGCAGTACATCCATACGGAAGCTT	118
TIMP3PCR29	
TIMP3clone7	erGluSerLeuCysGlyLeuLysLeuGluValAsnLysTyrGlnTyrLeuLeuThrGlyA CCGAGAGTCTCTGTGGCCTTAAGCTGGAGGTCAACAAGTACCAAGTACCTGCTGACAGGTC	85 600
TIMP3clone2	CCGAGAGTCTCTGTGGCCTTAAGCTGGAGGTCAACAAGTACCAAGTACCTGCTGACAGGTC	280
TIMP3HCM-3	CCGAGAGTCTCTGTGGCCTTAAGCTGGAGGTCAACAAGTACCAAGTACCTGCTGACAGGTC	178
TIMP3PCR29	
TIMP3clone7	rgValTyrAspGlyLysMetTyrThrGlyLeuCysAsnPheValGluArgTrpAspGlnL GCGTCTATGATGGCAAGATGTACACGGGGCTGTGCAACTTCGTGGAGAGGTGGGACCAGC	105 660
TIMP3clone2	GCGTCTATGATGGCAAGATGTACACGGGGCTGTGCAACTTCGTGGAGAGGTGGGACCAGC	340
TIMP3HCM-3	GCGTCTATGATGGCAAGATGTACACGGGGCTGTGCAACTTCGTGGAGAGGTGGGACCAGC	238
TIMP3PCR29	
TIMP3clone7	euThrLeuSerGlnArgLysGlyLeuAsnTyrArgTyrHisLeuGlyCysAsnCysLysI TCACCCCTCTCCCAGCGCAAGGGCTGAACATCGGTATCACCTGGGTTGTAAGTGCAAGA	125 720
TIMP3clone2	TCACCCCTCTCCCAGCGCAAGGGCTGAACATCGGTATCACCTGGGTTGTAAGTGCAAGA	400
TIMP3HCM-3	TCACCCCTCTCCCAGCGCAAGGGCTGAACATCGGTATCACCTGGGTTGTAAGTGCAAGA	298
TIMP3PCR29	

FIG. 16C

TIMP3clone7	leLysSerCysTyrTyrLeuProCysPheValThrSerLysAsnGluCysLeuTrpThrA TCAAGTCCTGCTACTACCTGCCCTGCTTGACTTCCAAGAACGAGTGTCTCTGGACCG	145 780
TIMP3clone2	TCAAGTCCTGCTACTACCTGCCCTGCTTGACTTCCAAGAACGAGTGTCTCTGGACCG	460
TIMP3HCM-3	TCAAGTCCTGCTACTACCTGCCCTGCTTGACTTCCAAGAACGAGTGTCTCTGGACCG	358
TIMP3PCR29CTCTGGACCG	10
TIMP3clone7	spMetLeuSerAsnPheGlyTyrProGlyTyrGlnSerLysHisTyrAlaCysIleArgG ACATGCTCTCCAATTTCGGTTACCCCTGGCTACCAGTCCAAACACTACGCCCTGCATCCGGC	165 840
TIMP3clone2	ACATGCTCTCCAATTTCGGTTACCCCTGGCTACCAGTCCAAACACTACGCCCTGCATCCGGC	520
TIMP3HCM-3	ACATGCTCTCCAATTTCGGTTACCCCTGGCTACCAGTCCAAACACTACGCCCTGCATCCGGC	418
TIMP3PCR29	ACATGCTCTCCAATTTCGGTTACCCCTGGCTACCAGTCCAAACACTACACATGCTCGCCCA	70
	Thr SerProS	
TIMP3clone7	InLysGlyGlyTyrCysSerTrpTyrArgGlyTrpAlaProProAspLysSerIleIleA AGAAGGGCGGCTACTGCAGCTGGTACCGAGGATGGCCCCCCCCGGATAAAAGCATCATCA	185 900
TIMP3clone2	AGAAGGGCGGCTACTGCAGCTGGTACCGAGGATGGCCCCCCCCGGATAAAAGCATCATCA	580
TIMP3HCM-3	AGAAGGGCGGCTACTGCAGCTGGTACCGAGGATGGCCCCCCCCGGATAAAAGCATCATCA	478
TIMP3PCR29	GCCACCCCCCGCACCGCTCCG.....erHisProArgThrArg	130
TIMP3clone7	snAlaThrAspProEnd ATGCCACAGACCCCTGAGGCCAGACCCCTGCCACCTCACTTCCCTCCCTCCGCTGA	205 960
TIMP3clone2	ATGCCACAGACCCCTGAGGCCAGACCCCTGCCACCTCACTTCCCTCCCTCCGCTGA	640
TIMP3HCM-3	ATGCCACAGACCCCTGAGGCCAGACCCCTGCCACCTCACTTCCCTCCCTCCGCTGA	538
TIMP3PCR29	190
TIMP3clone7	GCTTCCCTTGGACACTAACACTCTTCCCAGATGATGACAATGAAATTAGTGCCTGTTTCTT	1020
TIMP3clone2	GCTTCCCTTGGACACTAACACTCTTCCCAGATGATGACAATGAAATTAGTGCCTGTTTCTT	700
TIMP3HCM-3	GCTTCCCTTGGACACTAACACTCTTCCCAGATGATGACAATGAAATTAGTGCCTGTTTCTT	598
TIMP3PCR29	250

FIG. 16D

TIMP3clone7	GCAAATTTAGCACTTGGAACATTTAAAGAAAGGTCTATGCTGTCATATGGGGTTATTGG	1080
TIMP3clone2	GCAAATTTAGCACTTGGAACATTTAAAGAAAGGTCTATGCTGTCATATGGGGTTATTGG	760
TIMP3HCM-3	GCAAATTTAGCACTTGGAACATTTAAAGAAAGGTCTATGCTGTCATATGGGGTTATTGG	658
TIMP3PCR29	310
TIMP3clone7	GAACATCCTCCTGGCCCCACCCCTGCCCTTCTTTTGTTTGACATCATTCATTTCCA	1140
TIMP3clone2	GAACATCCTCCTGGCCCCACCCCTGCCCTTCTTTTGTTTGACATCATTCATTTCCA	820
TIMP3HCM-3	GAACATCCTCCTGGCCCCACCCCTGCCCTTCTTTTGTTTGACATCATTCATTTCCA	718
TIMP3PCR29	370
TIMP3clone7	CCTGGGAATTCTGGTGCCATGCCAGAAAGAATGAGGAACCTGTATTCCCTCTTCCTCGTG	1200
TIMP3clone2	CCTGGGAATTCTGGTGCCATGCCAGAAAGAATGAGGAACCTGTATTCCCTCTTCCTCGTG	880
TIMP3HCM-3	CCTGGGAATTCTGGTGCCATGCCAGAAAGAATGAGGAACCTGTATTCCCTCTTCCTCGTG	778
TIMP3PCR29	430
TIMP3clone7	ATAATATAATCTCTATTTTTTAGGAAAAA.....	1260
TIMP3clone2	ATAATATAATCTCTATTTTTTAGGAAAACAAAAATGAAAACACTACTCCATTTCAGGATT	940
TIMP3HCM-3	ATAATATAATCTCTATTTTTAGGAAAAA.....	838
TIMP3PCR29	490
TIMP3clone7	1282
TIMP3clone2	GTAATTCCCAACACCACCTGCT	962
TIMP3HCM-3	860
TIMP3PCR29	512

FIG. 17

